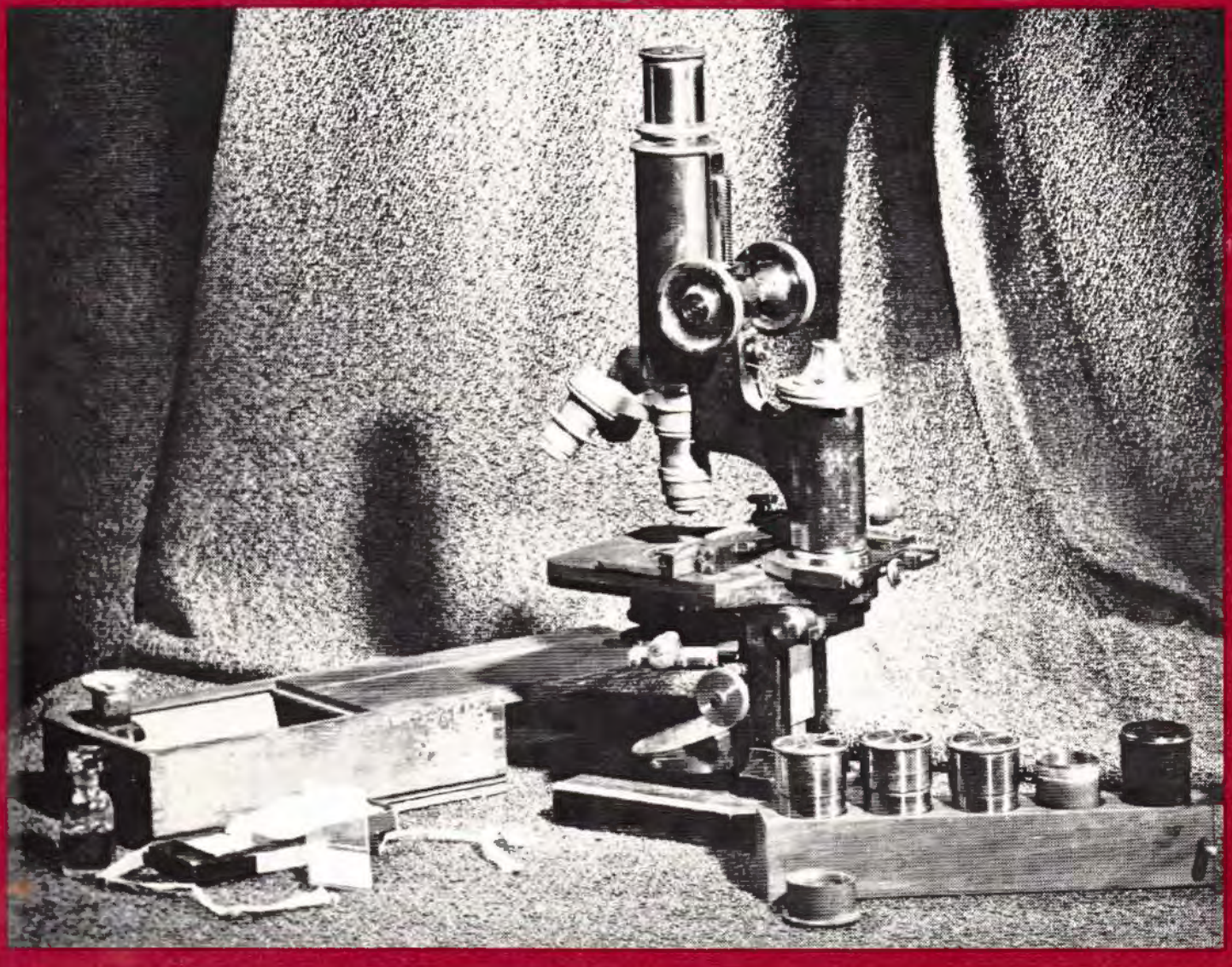
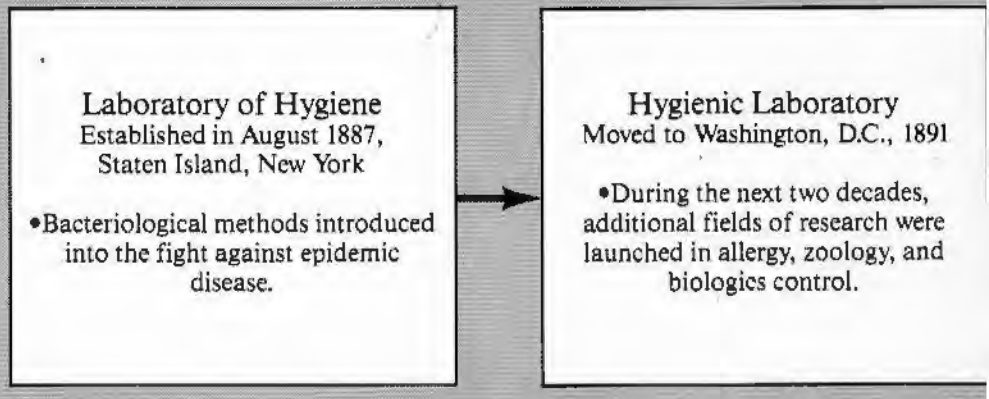
National Institute of
Allergy and Infectious Diseases

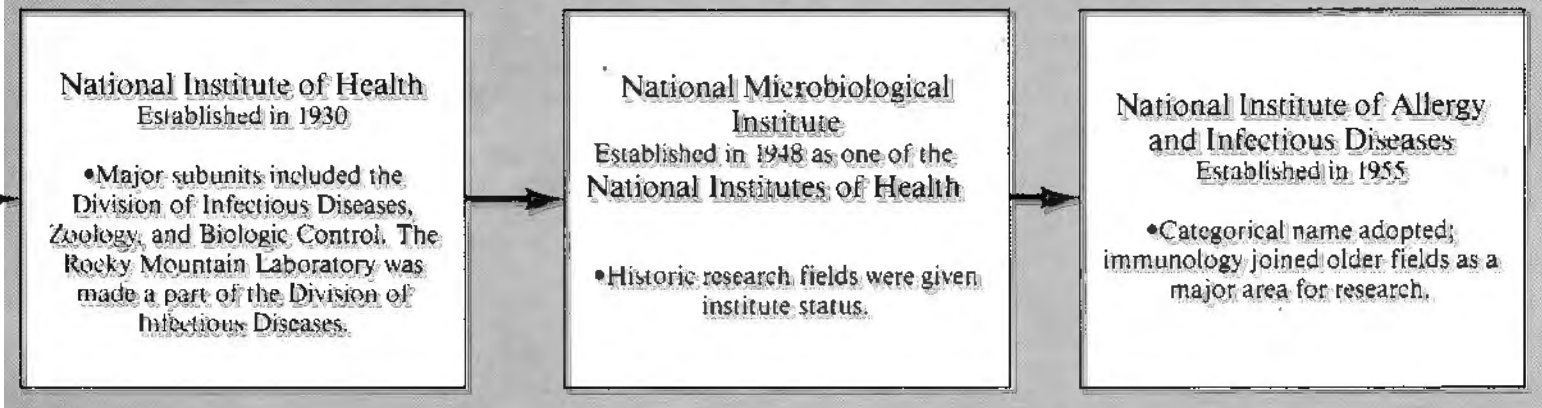
Intramural Contributions, 1887-1987



U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health



Cover illustration:
Zeiss microscope purchased in 1887 for United States Hygienic Laboratory and used by the Laboratory's first Director, Dr. Joseph J. Kinyoun.


National Institute of Health
Established in 1930
•Major subunits included the Division of Infectious Diseases, Zoology, and Biologic Control. The Rocky Mountain Laboratory was made a part of the Division of Infectious Diseases.
National Microbiological Institute
Established in 1948 as one of the National Institutes of Health
•Historic research fields were given institute status.
National Institute of Allergy and Infectious Diseases
Established in 1955
•Categorical name adopted; immunology joined older fields as a major area for research.

National Institute of
Allergy and Infectious Diseases

Intramural Contributions, 1887-1987

Edited by Harriet R. Greenwald and Victoria A. Harden

U.S. DEPARTMENT OF HEALTH AND
HUMAN SERVICES
Public Health Service
National Institutes of Health
National Institute of Allergy and Infectious Diseases
October 1987

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Foreword

Once every hundred years it is not unreasonable to assess where you have been, pat yourself on the back, and hope things will go as well in the next century. The NIH Centennial festivities have at their roots the NIAID Intramural Research Program, hence special emphasis on NIAID is appropriate during this year. This commemorative book represents a sampling of important contributions during the first hundred years of NIAID's Intramural Research Program, as seen through the eyes of several key participants.

We were very fortunate to recruit Victoria Harden, who, with her rich experience as a medical historian and particular knowledge of NIAID, immediately added reliability and a touch of class to the project. Her assistant, Harriet Reif Greenwald, put in long hours in the library searching for old references and arranging the significant events into their proper sequence. The editors recognized the need to assemble a panel of former NIAID investigators and administrators who could lend experience and expertise to this endeavor. This distinguished group consisted of Margaret Pittman, Dorland J. Davis, Leon Jacobs, Roger M. Cole and Sheldon G. Cohen. Except for Dr. Cohen, who is presently on the NIAID extramural staff and was assisted by William R. Duncan, these individuals came out of retirement to help plan this book and to provide a perspective on the past not available to most of us who participate in current NIAID events.

Evaluating a century of intramural research proved a daunting task. At the outset, the planning committee solicited suggestions of significant papers published by intramural researchers. Once the list was compiled—and it proved long, indeed—the committee divided the work into five major areas of research: bacteriology and mycology, parasitic diseases, viral diseases, regulation of biologic products, and immunology. One early classic paper from each area was selected to be reprinted, and members of the committee volunteered to write bibliographic essays for each area, generally focusing on work before 1970. For the more recent period, current laboratory chiefs were invited to submit annotated bibliographies that suggested the direction in which Institute research has proceeded. A brief historical chronology of each laboratory was developed to accompany each section. Selected photographs were obtained from NIAID files, from the National Library of Medicine, and from personal collections to enhance the text.

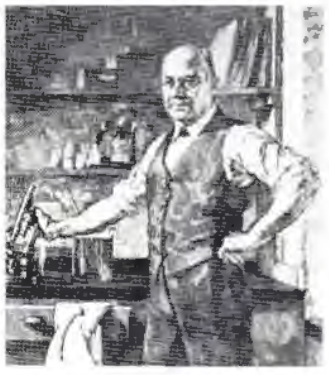
The hard work and long hours of preparing this book were tempered by enjoyable interchanges about research accomplishments, past and present. We hope that NIAID alumni and friends will derive similar pleasure from its pages. As we look to the future, equipped with exciting and sophisticated technological advances that have emerged from the past century of biomedical research, we also hope that 100 years from now a similar retrospective will chronicle even more impressive contributions to human health.

John I. Gallin, M.D.
Director, Intramural Research Program
National Institute of Allergy and Infectious Diseases

National Institute of Allergy and Infectious Diseases Intramural Chronology, 1887-1987

1st Period: Hygienic Laboratory, 1887-1930

1887
In August a Laboratory of Hygiene was established at the Marine Hospital on Staten Island, New York by Dr. Joseph J. Kinyoun for the purpose of bringing the then-new science of bacteriology to the study of epidemic diseases. The Marine Hospital Service was responsible for diagnosing infectious diseases among passengers on incoming ships to prevent the entry of disease into the United States. The bacteriological laboratory aided clinical diagnosis. Dr. Kinyoun served as Director until 1899.





1891
The Hygienic Laboratory, as it came to be called, was moved to the Butler Building in Washington, D.C.



1899
Dr. Milton J. Rosenau was appointed as Director of the Laboratory and served until 1909.



1900
Dr. Rosenau began publication of the Hygienic Laboratory *Bulletin*.



1901
Congress appropriated $35,000 to build a new building for the Hygienic Laboratory and, somewhat after the fact, authorized it to investigate "infectious and contagious diseases and matters pertaining to the public health."

1902
A reorganization act changed the name of the Service from "Marine Hospital Service" to "Public Health and Marine Hospital Service." Four new divisions were established in the Hygienic Laboratory: 1) Pathology and Bacteriology, 2) Zoology, 3) Pharmacology, and 4) Chemistry.

1902
President Theodore Roosevelt signed the Biologics Control Act. The function of testing as well as regulating all vaccines and other biologic products was delegated to the Division of Pathology and Bacteriology of the Hygienic Laboratory.



1902
In response to a request from the state of Montana, cooperative research on Rocky Mountain spotted fever was begun.



1904
The Laboratory occupied a new building on a five-acre tract at 25th and E Streets, N.W., Washington, D.C.



1905
Congress belatedly approved publication of the Hygienic Laboratory *Bulletin*.

1909
Dr. John F. Anderson was appointed Director of the Laboratory and served until 1915.



2nd Period: National Institute of Health, 1930-1948

1912
The name of the Service was shortened to "Public Health Service." The research program of the Hygienic Laboratory was broadened to include noncontagious diseases and water pollution.

1915
Dr. George W. McCoy was appointed fourth Director of the Laboratory and served until 1937, making his tenure the longest of all Directors to date.



1921
The Rocky Mountain Spotted Fever Laboratory was established in an abandoned school building in Hamilton, Montana, as a Pnblic Health Service field station.



1930
The Ransdell Act transformed the Hygienic Laboratory into the National Institute of Health (NIH).



1931
Congress purchased the Rocky Mountain Spotted Fever Laboratory in Hamilton, Montana.

1937
National Institute of Health was reorganized into eight divisions. The Divisions that evolved into NIAID or its predecessor NMI were: Infectious Diseases, Zoology, and Biologics Control. The Rocky Mountain Laboratory became a part of the Division of Infectious Diseases.



DBC

1937
Dr. Lewis R. Thompson was appointed Director of NIH and served until 1942.



1938-41
The National Institute of Health moved to its Bethesda, Maryland campus.



1939
The Public Health Service was transferred from the Treasury Department to the Federal Security Agency, the predecessor of Department of Health and Human Services (DHHS).

1942
Dr. R. Eugene Dyer was appointed Director of NIH and served through 1948.





3rd Period: National Microbiological Institute, 1948-1955

1948
The National Microbiological Institute (NMI) was established November 1. Constituent parts were the Laboratory of Infectious Diseases, Laboratory of Tropical Diseases, Laboratory of Biologics Control, and the Rocky Mountain Laboratory. Dr. Victor H. Haas was appointed Director.



1953
The NIH Clinical Center opened, and the NMI Laboratory of Clinical Investigation was established.





4th Period: National Institute of Allergy and Infectious Diseases 1955-present

1955
In June the Laboratory of Biologics Control in NMI was assigned to a newly established Division of Biologics Standards at NIH. Dr. Roderick Murray was appointed director.



1955
On December 29, the National Institute of Allergy and Infectious Diseases (NIAID) was established, replacing the National Microbiological Institute (NMI). Dr. Victor H. Haas continued to serve as Director until 1957.

1956
Because of expansion in the Institute, the new position of Associate Director in charge of research was created, and Dr. Dorland J. Davis was appointed to the post, which he held until 1964. From 1962-1964 the position was known as Director of Intramural Research.



1957
Dr. Justin M. Andrews was appointed Director of NIAID. He served until 1964.



1957
An intramural Laboratory of Immunology was established to meet the growing need for research on the mechanisms of allergy and immunology.



1957
The Middle America Research Unit was established in the Canal Zone jointly by NIAID and the Walter Reed Army Institute of Research.

1958
The Laboratory of Bacterial Diseases was established and continued until Dr. Norman B. McCullough retired in 1968.

1959
The Institute was reorganized to realign the larger laboratories with current research endeavors. The Laboratory of Tropical Diseases was abolished and replaced with four new laboratories: the Laboratory of Parasite Chemotherapy, the Laboratory of Parasitic Diseases, the Laboratory of Tropical Virology, and the Laboratory of Germfree Animal Research. From two of the larger segments of the Laboratory of Infectious Diseases, the Laboratory of Cell Biology and the Laboratory of Biology of Viruses were created. In 1986 the Laboratory of Biology of Viruses was disestablished when Dr. Norman P. Salzman retired.

1961
The Laboratory of Cell Biology was incorporated into the Laboratory of Biology of Viruses.

1964
Dr. Dorland J. Davis was appointed Director of NIAID. He served until 1975.



1965
Dr. John R. Seal was appointed Director for Intramural Research. The title of this position was changed to Scientific Director in 1969. He continued to serve until 1975, when he became Deputy Director of the Institute, a post he held until 1981.



1967-69
During these years, internal reorganization occurred. In 1967, the Laboratory of Microbiology was established. In 1968, the Laboratory of Infectious Diseases was split into two laboratories: the Laboratory of Viral Diseases and the Laboratory of Infectious Diseases. A name change occurred when the Laboratory of Germfree Animal Research became the Laboratory of Microbial Immunity. The Laboratory of Tropical Virology was disbanded in 1967. The Laboratory of Parasitic Diseases and the Laboratory of Parasite Chemotherapy were integrated into one Laboratory of Parasitic Diseases in 1969.







1972
The Division of Biologics Standards was transferred from NIH to the Bureau of Biologics in the Food and Drug Administration, but the Bureau's facilities were still maintained on the NIH campus.

1972
NIAID's portion of the Middle America Research Unit program was transferred to Gorgas Memorial Institute in the Republic of Panama.

1973
The Laboratory of Streptococcal Diseases was established and continued until Dr. Roger M. Cole's retirement in 1981.

1975
Dr. Richard M. Krause was appointed Director of NIAID. He served until 1984.



1977
The Laboratory of Immunogenetics was established.

1977
Dr. Kenneth W. Sell was appointed Scientific Director. He served until 1985.



1978
The first maximum containment facility (P4), for recombinant DNA research was opened at Fort Detrick, in Frederick, Maryland.



1979
The Rocky Mountain Laboratory was reorganized and renamed the Rocky Mountain Laboratories. The new laboratories created were: Laboratory of Persistent Viral Disease, Laboratory of Microbial Structure and Function, and the Epidemiology Branch.

1980
The Laboratory of Immunoregulation was established.



1981
The Laboratory of Molecular Microbiology was established.

1984
Dr. Anthony S. Fauci was appointed Director of NIAID.



1985
The Laboratory of Immunopathology was established.



1985
The Laboratory of Pathobiology at Rocky Mountaiu Laboratories succeeded the Epidemiological Branch.

1985
Dr. John I. Gallin was appointed Director, Intramural Research Program.



1987
The Laboratory of Cellular aud Molecular Immunology was established.

1987
The Biological Resources Branch was established to meet the increasing need for sophisticated technology by IRP scientists.

1987
AIDS-IRP Vaccine Development and Treatment Center established to meet the need for a coordinated AIDS effort by IRP scientists.

National Institutes of Health Scientists With Eponyms

Laboratory and Dates of Appointments

John F. Anderson, M.D. PHS, 1898-1915 Director, Hygienic Laboratory 1909-1915	*Dermacentor andersoni,* a wood tick, vector of Rocky Mountain spotted fever in the west.
E. John Bell, Ph.D. RML 1948-1972	*Rickettsia belli,* a rickettsia that appears to be nonpathogenic to humans.
Elmer G. Berry, Ph.D. LPD 1945-1963	*Tropicorbis berryi,* a potential intermediate snail host of *Schistosoma mansoni.*
Sara E. Branham, M.D., Ph.D. LBC 1928-1958	*Branhamnella catarhalis,* a bacterium found in the throat.
James Brennan, Ph.D. RML 1944-1974	*Brennania* sp., a genus of deer flies. *Chrysops clavicornis brennani,* a biting fly.
Willy Burgdorfer, Ph.D. RML 1952-1986	*Borrelia burgdorferi,* the cause of Lyme disease.
Herald R. Cox, Sc.D. RML 1936-1942	*Coxiella burneti,* the cause of Q fever.
G. Robert Coatney, Ph.D., Sc.D. LPC 1942-1966	*Plasmodium coatneyi,* a species of primate malaria. *Phyllodistomum coatneyi,* a helminth.
R.A. Cooley, Sc.D. RML 1928-1946	*Haemaphysalis cooleyi,* a tick.
Chester W. Emmons, Ph.D. LID 1936-1967	*Emmonsiella capsulatum,* sexual stage of a fungus causing histoplasmosis. *Emmonsia parva,* a fungus of rodents. *Emmonsia crescens,* a fungus of rodents.
Don E. Eyles, Sc.D. LPC 1943-1963	*Plasmodium eylesi,* a species of primate malaria.
Edward Francis, M.D. Hygienic Laboratory 1919-1936	*Francisella tularense,* the cause of tularemia.
Leon Jacobs, Ph.D. LPD 1937-1979	*Simulium jacobsi,* a tropical fly.
Geoffrey M. Jeffery, Sc.D. LPC 1944-1969	*Plasmodium jefferyi,* a species of primate malaria.
William L. Jellison, Ph.D. RML 1929-1962	*Jellisonia* sp., a genus of fleas. *Besnoitia jellisoni,* a protozoan. *Chrysozona jellisoni,* a biting fly. *Tabanus stonei jellisoni,* a horsefly.

Glen M. Kohls, Sc.D. RML 1931-1969	*Kohlsia* sp., a genus of fleas. *Croypsylloides kohlsi,* a flea. *Clubiona kohlsi,* a spider. *Strebla kohlsi,* a biting fly. *Ploceophilus kohlsi,* a cockroach. *Kohlsiella* sp., a subgenus of ticks. *Ixodes kohlsi,* a tick. *Haemophysalis kohlsi,* a tick. *Boophilus kohlsi,* a tick. *Neoschongastia kohlsi,* a mite. *Trombicula kohlsi,* a mite. *Helenicula kohlsi,* a mite.
Ralph R. Parker, Ph.D. Director, RML 1921-1949	*Rickettsia parkeri,* a rickettsia that appears to be nonpathogenic for humans. *Ornithodorus parkeri,* a tick. *Meringis parkeri,* a flea. *Borrelia parkeri,* a spirochaete.
Cornelius B. Philip, Ph.D. RML 1930-1970 Director, 1962-64	*Amblyomma philipi,* a tick. *Ixodes philipi,* a tick. *Culex philipi,* a mosquito. *Philipotabanus* sp., a genus of flies. *Anerythrops philipi,* a fly. *Bolbodimyia philipi,* a fly. *Cydistomyia philipi,* a fly. *Haematopota philipi,* a fly. *Dissimas philipi,* a fly. *Silvius philipi,* a fly. *Leucotabanus cornelianus,* a fly.
Martin D. Young, Sc.D. LPC 1937-1962	*Plasmodium youngi,* a species of primate malaria.

Abbreviation Key

PHS Public Health Service
LBC Laboratory of Biologics Control
LPD Laboratory of Parasitic Diseases
RML Rocky Mountain Laboratory
LID Laboratory of Infectious Diseases
LPC Laboratory of Parasite Chemotherapy

Prepared by Dorland J. Davis, M.D.
Director, NIAID 1964-1975

Part I.
Intramural Contributions:
A Retrospective View

repeated currents had been used through large uninsulated needles. The result was a circular raw mass, projecting above the surface, which refused to heal. Myxo-sarcomas act badly under electrolysis. Around the point of entrance of the needles slight ulceration at first appears, and subsequently unsightly fungoid growths. The treatment in these cases undoubtedly tends to hasten rather than to retard their progress. Sarcomas do not ulcerate so readily, but they can not be treated with the same freedom as scirrhus.

Fatty tumors can be treated by electro-puncture and by very strong currents, not only without bad effects, but with the prospect of very materially lessening their size in many cases, and occasionally causing them to entirely disappear. Fatty tumors are, of course, very bad conductors—much worse than the hard fibroids which electrolyze so slowly—yet they do disappear.

This fact leads me, in conclusion, to refer to the two methods of action through which we obtain results from electrolysis. The first and most apparent is the absolute destruction of tissue which takes place at the time of treatment. It is unnecessary for me to enter into the physical details preceding and accompanying the destructive action of the galvanic current. It is sufficient to say that a molecular separation takes place, more or less marked, according to the density or softness and fluidity of the tissues. Some suppuration may follow, and thus, by an actual loss of substance apparent to the sight, the tumor decreases in size. If, however, these were the only active forces in the electrolytic process, the method would lose much of its effectiveness. If this be not so, how can we account for the many well-attested cases where morbid growths have entirely disappeared under simple external applications? Herein is the difference between the electrolysis of organic and inorganic substances. In the electrolysis of inorganic substances the effects cease as soon as the current ceases, the substances remaining in the condition that the current left them. The electrolysis of organic substances, on the contrary, starts a process that continues long after the current ceases to flow. Besides this subsequent effect, this retrograde metamorphosis, as it has been termed, the current penetrates the tissues, and induces various important changes beyond and beneath the eschar, and these combined agencies do far more in many cases to diminish the size of morbid growths and prevent further development than an actual destruction of a limited area.

OBSERVATIONS ON THE CHOLERA BACILLUS AS A MEANS OF POSITIVE DIAGNOSIS.

By S. T. ARMSTRONG, M. D., and J. J. KINYOUN, M. D.,
UNITED STATES MARINE HOSPITAL SERVICE.

On September 24th it was reported in the public press that the steamship Alesia had arrived at the port of New York on the previous day, having had 609 immigrant passengers on board, of whom eight had died of cholera *en voyage*, and several suffering from that disease had been admitted to the Swinburne Island quarantine hospital on arrival. According to the captain's report, the first death had occurred September 12th, the rest of the deaths occurring and cases developing within eleven days. Subsequently new cases, some fatal, occurred.

At the request of the writers, the health officer, Dr. William M. Smith, accorded the privilege of visiting the hospital, in order to examine the patients and secure material for bacteriological investigation. The patients were first seen by the writers on October 3d, when cultivations were made from excreta obtained at that visit. Plate cultivations were made on the 5th, and characteristic colonies of comma bacilli were found on the 6th, conforming in every particular to the description given by Koch. On October 9th it was reported in the public press that Dr. Shakespeare, of Philadelphia, had, from inoculations made by him from excreta obtained from these patients, also found the comma bacillus.

On October 13th the steamship Britannia, belonging to the same company as the Alesia and coming from the same points in Italy, arrived at this port with 406 passengers on board. *En voyage* there were three deaths of emigrants; the diagnosis, though pointing to intestinal trouble, Health Officer Smith considered obscure, and held the vessel in quarantine.

On October 17th Dr. Smith requested the writers to visit the quarantine hospital with him, in order "to take some specimens from a suspicious case for cultivation."

The visit was made on the morning of the 18th; the case was that of a boy five years old, in collapse at the time of the visit. Cultivation tubes were inoculated from washings from the bowels, as there were no fæcal discharges available. A visit was again made in the afternoon in order to be present at the necropsy, the child having died at two o'clock. No pathological lesions were presented; the small intestine contained liquid fæces, with which cultivation tubes were inoculated. The necropsy was made five hours after death.

Plate cultivations were made from the tubes as soon as possible, and on the evening of the 19th Dr. Smith was informed that the comma bacillus was present in the specimen, but a positive diagnosis would not be given until the plate cultivations had fully developed. At Dr. Smith's request, two inoculated cultivation tubes were given to him: these, it was subsequently learned, were submitted to Dr. H. M. Biggs, Director of the Carnegie Laboratory, and Dr. T. Mitchell Prudden, Director of the Alumni Laboratory of the College of Physicians and Surgeons, for examination. On the morning of October 20th, typical colonies of comma bacilli had developed on our plate cultivations. On October 22d, at a conference with Dr. Smith, Dr. Biggs, and Dr. Prudden, it was learned that their cultivations had afforded the same results. Consequently the existence of cholera on the steamship Britannia was demonstrated by bacteria investigations.

On October 24th the hospital was again visited on account of the death of a man aged fifty years. He was taken ill, with diarrhœa and vomiting, on the night of the 22d and died on the morning of the 24th. The necropsy was made eleven hours post mortem; no pathological lesions were presented, excepting a slight congestion of the intes-

A Note on the Reprinted Papers

The authors of the bibliographic essays chose one classic paper that helped to launch the area of research each was addressing. Because two of the reprinted papers were excessively long, sections containing supporting data were omitted. Other papers are reprinted in their entirety.

The authors of tbe reprinted papers are pictured here.



Samuel T. Armstrong



Joseph J. Kinyoun



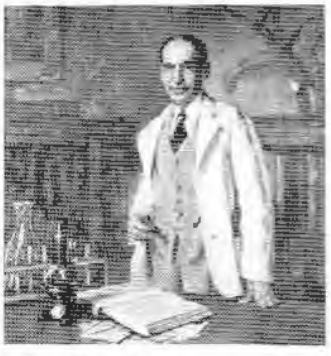
Charles W. Stiles



John F. Anderson



Wade H. Frost



Milton J. Rosenau

tines, and the contents of the latter were fluid. Inoculations of cultivation tubes were made, and transferred to the third and fourth dilutions: plate cultivations were made from these, and thirty hours later the characteristic colonies of the comma bacillus were abundant.

On the 25th one of the writers was placed by Dr. Smith in charge of the patients at the quarantine hospital. A woman, aged about twenty-two years, who had been removed from the Britannia on the evening of the 24th, was in the hospital, suffering from an acute attack of cholera. A nursing child, about one year and a half old, occupied the same bed with her. Her case was so characteristic that no cultivations were made from her excreta. Under treatment she recovered, and the child has not been ill up to date.

On the 27th a boy, six years old, died suddenly on the Britannia. His mother stated that he had slight fever and vomiting for three days previous to his death. The necropsy was made twenty-four hours post mortem. No pathological feature presented, excepting an ascaris protruding from the anus, with a mass of ascarides in the cæcum, the small intestines containing white, glairy mucus. It was regretted that no cultivation tubes were at hand, and consequently inoculations were not made. But the case presented the same appearance as those reported above.

On the 28th a child, five months old, died in its mother's arms while being transferred from the vessel. She stated that the child had been ill for several days, but had no diarrhœa. The necropsy was made twenty hours post mortem. No pathological features were apparent. The small intestine contained liquid fæces; the colon, apparently healthy fæcal matter. Inoculations of cultivation tubes were made, and, after being delayed twenty-four hours, specimens were taken therefrom and examined, the cholera bacillus being found in great numbers: in fact, so plenteously, and unmixed with other organisms, that it was not considered necessary to make a plate cultivation.

No cases nor sudden deaths have occurred since that date, and here we rest the case.

The deductions which may be derived from these observations are as follows:

1. That in deaths among emigrants coming from a cholera-infected district, a necropsy is absolutely essential, and cultivation tubes should be inoculated with the contents of the intestine, for the purpose of determining the cause of death.

2. That successful inoculations may be made at least twenty-four hours after death.

3. As the symptoms, in the cases examined, were by no means always well defined, the examinations were confirmatory evidence of the value of bacteria cultivation as a means of positive diagnosis.

November 6, 1887.

The Academy of Medicine and the Board of Health.—Following the reading of Dr. Bryant's paper, an account of which we give in this issue, Dr. Ellsworth Eliot moved the appointment of a conference committee of five, including the president of the Academy, to co-operate with the board of health. The motion was carried.

REPORT OF A CASE OF TUBERCULAR PHTHISIS TREATED WITH THE PNEUMATIC CABINET.

By WILLIAM B. WOOD, M. D.

This paper does not enter the field in which the pneumatic-cabinet treatment has yielded the most valuable results—which is in the control and cure of the earlier stages of pulmonary affections—but is simply a record of what it is possible to do with the graver forms of established pulmonary diseases, to give relief and comfort, and to at least prolong life; not merely to extend an existence of chronic invalidism, but frequently to prolong life with such improvement of health as enables a patient to resume the regular daily occupations.

On the 22d of April, 1887, a gentleman, about forty years of age, presented himself at my office for examination. He had just returned from a winter in Florida, where he had experienced steady, and for two months rapid, decline. His case proved to be a typical one of tubercular phthisis. Every one of five specimens of sputum examined contained bacilli in very unusual numbers. There were broncho-vesicular respiration in the right lung from the third interspace to the apex; crepitant râles in the clavicular region; moist râles and softening at the apex. There were creaking friction-sounds: the percussion notes were those of thickened and adherent pleuræ, with some consolidation: there were also infra- and supra-clavicular retraction. In the left lung crepitant râles were heard from the clavicle to the apex. The rational symptoms were such as always accompany the foregoing physical signs: restless nights, racking cough, alternate hectic and clammy stages, the septic expression and complexion, shortness of breath, lack of appetite, loss of digestive and assimilative power, with daily expectoration of from eight to ten ounces, and a progressive loss of vitality, weight, and lung.

At this time the patient was unable to accomplish normal respiration with sufficient vigor to meet the natural demands of waste-repair and up-building.

By normal respiration is meant, of course, the act of filling the lungs in inspiration with pure air and deoxygenated blood, and in expiration that of emptying them of deoxygenated air and oxygenated blood, thus supplying red blood to medulla, mesentery, and mucous membranes. As I knew of but one process—the pneumatic differentiation—which could accomplish this, treatment in the cabinet was recommended as a means of temporary amelioration certainly, and possibly of permanent benefit.

The degree of success to be obtained with the pneumatic cabinet depends largely upon the individualization of the various cases and the fine adaptation of its functions to the age, sex, particular disease, and general physical condition of each patient. The personal equation of the operator is also a most important factor. The results to be obtained by the cabinet depend upon the operater's skill. It is a powerful instrument, but so mathematically exact that it may be perfectly controlled, while its several distinct and separate functions make it an instrument of great scope and power of combination; but those who expected the cabinet, *per se*, would work miracles, have been disap-

A Century of Research in Bacteriology and Mycology: Contributions of NIAID Intramural Scientists, 1887 to 1987

Roger M. Cole

I was somewhat bemused, but not surprised, to note that the program for the NIH Centennial Symposia contains no mention of bacteriology. Even in the infectious diseases section, the titles emphasize viruses and host defenses—a reflection of the times and current trends. The contributions of bacteriological science to genetics, molecular biology, and biochemistry are obscured in the burgeoning applications of these fields to problems closer to the biology of man, and unsolved non-viral infectious diseases are rare. It was not always so. One hundred years ago, initiating the span of research that we now celebrate, bacteriological diseases and their agents provided the impetus for establishment of the first research laboratory of the Public Health Service (then the Marine Hospital Service) and, eventually, for the evolution that resulted in today's National Institutes of Health.

One of the latter's current components, the National Institute of Allergy and Infectious Diseases (NIAID), is the direct descendant of Joseph Kinyoun's original Laboratory of Hygiene. Within it, through changes of name and emphasis, many findings made by its intramural scientists have been influential in the field of bacteriology and mycology and often, as a consequence, in medicine and public health. Certainly, not all outstanding discoveries were achieved by these workers—but many were, and it is some of these that we highlight here as part of the NIH Centennial celebration. (Only appropriate, considering the bacteriological origins of the whole thing!) However, since hundreds of studies have been carried out and published over these years, the selection of the relatively few that can be featured as examples is a difficult task. To facilitate this, I have used, as a guide, the designation of *landmark*—defined as "an event or development that marks a turning point or stage."

That seems reasonable enough, but the problem now becomes one of determining what constitutes a turning point. It is assumed that most findings that survive editorial reviews and thus find publication can be presumed (perhaps charitably, in some instances) to offer something new or different and so improve the body of science in one degree or another. But which of these have the consequences that let us recognize (sometimes retrospectively) that a turning point was reached or a stage passed? The judgment of this, despite any other knowledgeable inputs, must ultimately rest with the beholder. Therefore, to be quite clear at the outset, I outline the sorts of "turning points" that guided my choices for the accompanying bibliography.



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First and most obvious is the discovery and characterization of a new disease of humans. A second turning point is the finding of its etiology, or the etiology of a disease previously known only clinically or epidemiologically; without knowledge of the agent, the development of vaccines or specific therapy cannot even be attempted. The successful provisions of these measures are also turning points. Also important are epidemiological findings that define disease distribution, mode of transmission, vectors, reservoirs or alternate hosts, and the like—sometimes suggesting an etiology, if not already known. But all of these, in respect to bacterial etiologies, are mostly things of the past.(Or are they? Recall Legionnaire's Disease!) In any event, it is apparent that the character of bacteriological and mycological research has changed as emphasis has gradually shifted from disease discovery toward a greater concentration on basic studies of the microorganisms themselves. In addition to creating a broader literature in physiology and taxonomy, these expanding studies gave rise to today's predominant and growing fields of genetics and molecular biology. They also offer new potential "turning points," in instances when new

laboratory techniques or basic discoveries have given new directions to scientific thought, stimulated fruitful new avenues of research, or resulted in improved methods for such purposes as diagnosis, propagation of the microorganism, and vaccine production. Obviously, there is some overlap among these various categories. There are also problems in proper attribution (i.e., who found what first?), as well as uncertainties in citing a single publication when the impact of the ultimate discovery is really clarified by several. However, it is not my purpose here to settle controversies nor to investigate minutiae but rather to supply some idea of the scope and influence of 100 years of intramural studies in bacteriology and mycology.

The easiest to deal with are new diseases and disease etiologies. Although bacteriology was a relatively new science in 1887, such old and distinguishable diseases as cholera, diphtheria, tetanus, typhoid, gonorrhea, cerebrospinal meningitis, scarlet fever, lobar pneumonia, and tuberculosis were already known to be associated with distinct and microscopically visible microorganisms, each of which had already been named by European scientists (though many designations were subsequently revised—often many times). The tasks of Kinyoun's small Laboratory of Hygiene were oriented toward diagnosis, prophylaxis, and control. For example, the first diagnosis of cholera in the United States was made there in 1887.[1] Later (1908), McCoy[2] made the first demonstration of bubonic plague in California ground squirrels associated with a human case and thus initiated the recognition of rodents as a reservoir and source of disseminated infection. The laboratory had facilities for the diagnosis of plague (the organism had been isolated in Europe by 1894) and of diphtheria, and for production of vaccine for the former and antitoxin for the latter. However, as the staff and their successors practiced the methods of bacteriology as well as their clinical and epidemiological expertise, it was perhaps inevitable that they should find new diseases, new bacteria, and new hosts. The first and best known disease, now voluminously documented in all its aspects, was Rocky Mountain spotted fever (RMSF), delineated by Anderson in 1903.[3] Although its association with ticks was apparent, it was not until 1906 that transmission by infected ticks (to guinea pigs) was shown by King[4] and then by Ricketts. These studies introduced a long period of progress in what we now know to be diseases caused by *rickettsiae*—a field in which the contributions of PHS researchers became well known. For example, Anderson and Goldberger, in 1909[5], demonstrated that Mexican Typhus Fever was not RMSF (as had been previously suspected), but predicted its transmission by the body louse[6] as was demonstrated a few months later by Ricketts and Wilder, and showed the cross-immunity produced in monkeys by its agent and that of Brill's disease.[7] After the latter was shown to be a recrudescent form of the European or epidemic typhus, other workers used convalescent Brill's serum to show that the Mexican disease was also epidemic typhus. The typhus picture was further clarified by epidemiologic work of Maxcy in 1926:[8] despite his erroneous equation of Brill's Disease with



Dr. Cole in LID laboratory, 1953.

endemic typhus, he clearly showed that the latter was a distinct disease of the southeast U.S., with a rodent reservoir. Subsequently, the studies of Dyer, Badger, and others[9-11] demonstrated its transmission by the rat flea. The etiologic agent was later distinguished from that of epidemic typhus, aided by differential scrotal reactions of guinea pigs found in 1917 by Neill,[12] and was defined and named *Rickettsia typhi* (as distinguished from *R. prowazekii* of epidemic typhus) by Philip in 1943.[13]

The story of typhus, of whatever form, is a long one with a plethora of references, many of which made small but important contributions to ultimate understanding; but few of which constituted landmark studies in themselves. A sharper picture is presented by Q Fever. This began in the U.S. with the discovery by Davis and Cox, in 1938[14] of a new filter-passing agent from ticks, and the finding by Dyer[15] (from personal experience!) that the highly infectious agent caused a human disease like that just described as Q (Query) Fever by Derrick in Queensland, Australia. Derrick called the agent *Rickettsia burneti*, Cox labeled it *Rickettsia diaporica*,[16] and it eventually became the only member of a new genus, as *Coxiella burneti*.[17]

A classic example of elucidation of a new disease is that of rickettsialpox in New York. In a series of five papers in 1946 and 1947,[18-22] Huebner and colleagues described the disease, the agent, its reservoir in mice, and its accidental transmission to man by a rodent mite. Rarely are such landmark findings presented so clearly and compactly. It later became apparent that the disease is not a major nor frequent one, that it occurs sporadically in other parts of the world, and that the agent (*Rickettsia akari*) can be isolated from mice and mites in other regions of the U.S.A. as well as in Soviet

Union and Korea.

Another disease historically associated with the work of PHS scientists is tularemia. Elucidation of its etiology began in 1911-12, when McCoy and Chapin investigated a plague-like disease of ground squirrels[23,24] and described *Bacterium tularense*. It was not until two years later that Wherry and Lamb[25] showed that the bacterium (later named *Francisella tularensis*) could also cause disease in man. Francis extended the observations.[26,27] Many of his other studies clarified the nature of the agent, its distribution in animals, the participation of various arthropods such as ticks and deer flies as vectors, dermal and pulmonary routes of infection in man, and other aspects of tularemia.

Most of the foregoing examples concern new diseases for which the etiology was usually soon found. In other instances, PHS scientists uncovered the etiology of diseases that had been known for sometime but whose cause remained obscure. The oldest of these, pellagra, was long considered to be of obscure, infectious origin, but was shown by Goldberger in 1914[28] to result from a nutritional deficiency, thus, supplying a reverse example of the usual bacteria-oriented turning points considered here. On the other hand, psittacosis was believed to be a viral disease until Lillie, in the course of pathologic examination of infected humans and animals, found microscopically-visible rickettsia-like inclusions in 1930.[29] He named the organism *Rickettsia psittaci*, but it was later placed in the genus *Chlamydia*. Similarly, a virus was thought to be the cause of the predominant form of primary atypical pneumonia until 1962, when the "Eaton Agent" was first grown on an artificial medium and recognized as a mycoplasma by Chanock, Hayflick, and Barile.[30] It was designated as a new species and named *Mycoplasma pneumoniae* the next year.[31] It was the first recognized and remains the most prominent mycoplasmal cause of human disease. More recently (1974), a syndrome of skin lesions (erythema chronicum migrans) followed by chronic inflammatory arthritis was designated Lyme disease. Despite its clear association with ixodid ticks and numerous investigations, its etiology remained a mystery. The first evidence of its cause as a spirochete (since named *Borrelia burgdorferi* by others) resulted from the work of Burgdorfer and colleagues at the Rocky Mountain Laboratories, and elsewhere in 1982.[32] This study furnishes the most recent example of the discovery of a bacterial etiology for a recognized human disease, though it may not be the last.

Once the etiology of a disease was established, the usual next step considered was production of active immunity by a vaccine. This was especially true in the days before the advent of specific chemotherapies, and Rocky Mountain spotted fever is a prime example. Spencer and Parker, reasoning by analogy from results with typhus by European workers, produced a phenolized vaccine from ground infected ticks.[33] The vaccine was widely used and reasonably effective in Montana for 15 years.[34] However, production was laborious and somewhat uncertain. The need for a better and more reliable source of antigen in quantity was clear. The key was provided by the cultivation of the organism in chick embryos by Bengtson and Dyer in 1935,[35] followed by Cox's application of the method in yolk-sacs,[36] which permitted the growth of the rickettsiae of typhus and Q fever as well.[37] This led to mass production of rickettsial vaccines: indeed, during the years of World War II, the Rocky Mountain Laboratory became, in large part, a vaccine factory. The methods were licensed to commercial producers, but problems with efficacy of the RMSF vaccine led to cessation of production in 1978. Although the availability of effective antibiotics now decreases the need for a vaccine, problems with adequate and early diagnosis of RMSF continue to stimulate an interest in prophylaxis. Most recently, MacDonald and colleagues at RML[38] applied the tools of molecular genetics to expression and production in *E. coli* of a surface antigen of *Rickettsia rickettsii*, which they demonstrated to be protective in mice—thus offering a new potential for production of antigen and for immunization.

Among epidemiologic studies, that of Maxcy[8] which distinguished endemic from epidemic typhus and other rickettsial diseases, has been mentioned. Another field study that was influential was that of psittacosis in 1930 by Armstrong,[39] which resulted in governmental regulation of the importation of psttacine birds. Also outstanding are the studies of institutional outbreaks of Q fever at two different times and in two different sites at the NIH. These, carried out by different workers, defined not only epidemiologic features of the disease, but also noted clinical variations, confirmed the etiology, and emphasized the high infectivity and capacity for airborne transmission of this agent.[40-43] New potentials for transmission of the disease were revealed by the studies of Huebner, Bell, and associates in southern California,[44,45] emphasizing the hazards to human health of cattle and their milk infected with the Q fever organism. In another field, the findings of Emmons first pointed out the reservoirs of histoplasmosis in soil[46] and bats,[47] of coccidiomycosis in soil,[48] and of cryptococcosis in soil[49] and pigeon droppings[50]—thus supplying crucial information on sources of infection by these pathogenic fungi.

Basic laboratory findings, unless clearly related to such disease-oriented applications as vaccine or toxin production, are more difficult to assess as turning points. Not infrequently, their influence requires the passage of time for recognition and evaluation. An example is the first demonstration, by Somerson *et al.*, in 1967,[51] that *Mycoplasma pneumoniae* colonies adhere firmly to a glass surface during growth, thus supplying a concentrated mass of organisms that can be easily washed and later detached to supply antigen or organisms for a vaccine. Though originally employed to provide a clean (non-anti-complementary) antigen for complement fixation tests, the method has been tested with many mycoplasmas, and it became recognized that adherence is a characteristic that helps to differentiate some species from others. Complement fixation became supplemented (and later largely replaced) as a serologic test for mycoplasmas by simpler and more specific tests devised by Purcell, Taylor-Robinson, Wong, and Chanock in 1966. These metabolic inhibition tests, easily read and

now almost universally used, differ according to the metabolism of the mycoplasma—being adapted to acid-producing species,[52] non-acid-formers,[53] and urease producers.[54]

Basic studies with no relation to disease often have long-term reverberations, or the potential therefore. One example that comes easily to mind (and I include this with some diffidence) is work done in my laboratory between 1962 and 1966. This was the first to show a difference in modes of cell wall replication between gram positive and gram negative bacteria. By using a new approach of marking the walls of living bacteria with fluorescein-labeled antibodies, we showed that the walls of streptococci replicate in a discrete fashion[55] and those of gram negative bacteria like *Salmonella typhosa* and *Escherichia coli* are replicated in a diffuse mode.[56,57] Such differences were unknown and their demonstration stimulated a rebirth of research in this rather esoteric field that led to widespread application of electron microscopic and biochemical techniques. Results with cocci were rapidly confirmed. Despite many variations and ups and downs in the intervening 25 years, the most recent published results appear to confirm also the principle of diffuse wall replication in gram negative rod-shaped bacteria.

Scientific thought was also changed by new intramural discoveries in mycology. It was long accepted that the agents of the systemic mycoses, histoplasmosis and cryptococcosis, were *fungi imperfecti*. This concept was destroyed when Kwon-Chung demonstrated the sexual stage of *Histoplasma capsulatum* in 1972[58,59] and that of *Cryptococcus neoformans* in 1975,[60] necessitating the redesignation of these important pathogens in new genera *Emmonsiella* and *Filobasidiella* respectively. A study of the mating types of the former[61] showed that both (+) and (-) types were equally distributed among soil isolates but that the (-) type was 7 times more common in clinical isolates. The significance of this finding remains to be more fully explored, but its elucidation and exploration of the promising theoretical basis for genetic study have been hampered by loss of active mating capacities in isolates maintained in laboratory passage.

Basic research often produces unforeseen ramifications. One such example resulted from a need to identify a newly discovered microorganism found associated with a disease of citrus trees. Though previously cultured and suspected of being a mycoplasma, it was not characterized until collaborative work in 1973 by European and NIAID workers defined it as a new genus and species with a helical shape and rotary motility,[62,63] attributes previously unknown among mycoplasmas. The organism, named *Spiroplasma citri*, became the prototype of a new group of mycoplasmas, previously totally unsuspected but now found to be widespread in nature. In retrospect, it became apparent that some of these spiroplasmas, which had never been cultured or were considered to be spirochete, had been previously observed in a disease of corn, in fruit flies with a sex-ratio imbalance in the progeny, and in ticks. The definition of the first spiroplasma initiated a remarkable expansion of plant and insect pathology, and some dozen species and serovar groups are now known from ticks, bee diseases, beetles, and many other insects, and from plants, plant diseases and their insect vectors—with no end in sight. The fundamental and continuing contributions of Tully and collaborators to the understanding of these and other mycoplasmas are numerous and exemplary.

The foregoing examples were selected as representative of the several categories of "turning points" previously outlined. There are many other significant contributions by intramural researchers that do not fit precisely into predetermined slots. One of these is Armstrong's finding in 1925[64] that 25% of commercial bunion pads were contaminated with spores of *Clostridium tetani*, resulting in cases of human tetanus (about 80% fatal) when such pads were used, as was then the practice, as dressings following the scarification method of vaccination for smallpox. His recommendations resulted in the discontinuance of such dressings and, later, in general adoption of the multiple pressure method of vaccination as promulgated by Leake.[65]

Then, too, there are works which, taken in the aggregate, provided significant and essential knowledge of the subject area, often to the extent that it became lastingly associated with the name of the researcher. Many of these findings became best appreciated cumulatively and in retrospect. Because of this and of the volume of contributions over many years, I can include here only a few sample references that indicate, but by no means encompass, the field and directions of the researcher's work. One cannot, for example, overlook the pioneering contributions of Evans that, from 1918 on, led to differentiation of the *Brucella* species causing Malta Fever in man and Bang's Disease in cattle, established their occurrence in many mammalian species as well as in human disease, and progressively clarified the serology and classification of these organisms.[66-68] She also contributed extensively to the early understanding of streptococci and their bacteriophages, having initiated the collection and study of these bacteria in the Hygienic Laboratory. In similar fashion, the name of Branham is closely associated with early studies of the genus *Nesseria*, in which she illuminated the taxonomy, described a new species in meningitis, explored properties of immune sera, and, especially, distinguished the three basic serotypes of the meningococcus.[69,70] A third and widely recognized longtime association has been that of Pittman with the genus *Haemophilus* (and *Bordetella*) and with various aspects of pertussis—a field to which she continued to contribute after relocation from NIH to the FDA's Bureau of Biologics and even after official retirement.[71,72]

Other lasting associations of a researcher's name with a particular organism or disease are known. One need only recall (and I won't list them here) those intramural workers, already mentioned, who made continuing contributions, in more than the specific instances mentioned, to the elucidation of plague, tularemia, typhus, spotted fever, Q fever, rickettsialpox, psittacosis, mycoplasmal diseases, and the systemic mycoses. There is no space to list here, much less to even casually annotate, their numerous and collective works. Also missing, regrettably,

must be reference to the hundreds of productive contributions made by other intramural scientists who, in the aggregate and over the years, studied most organisms and aspects of bacteriology and mycology. But the scene has shifted and times have changed. Although mycological investigations continue here, no bacteriological laboratory now exists in NIAID at Bethesda. Such research, including investigations of pertussis, gonococci, spirochetes, rickettsiae, and related microorganisms, continues at the Rocky Mountain Laboratories in Hamilton, Montana. Studies of bacterial genetics and of mycoplasmas are located at the Fort Detrick facility in Frederick, Maryland, as outlying components of the Bethesda-based Laboratory of Molecular Microbiology. The latter name, unintendedly, but wryly, reflects recent trends that have engendered a current academic controversy over the potential loss of identity of microbiology as a separate discipline and stimulated fears (as expressed by one observer) of its "being smothered by molecular biology." (See *Letters*, pp. 355-56; and *Microbiology By Any Other Name*, pp. 373-74 in *ASM News*, Vol. 53, No. 7, July 1987). Whether this change has really taken place, or will do so, in NIAID and other research institutions as well as in academic situations is a matter for debate and continued observation of future trends. Clearly, it is unlikely that the halcyon days of "pure" bacteriology will recur. However, current and future emphasis in no way lessens the importance of the past 100 years of NIAID intramural contributions. May the next 100 years be as productive!

Roger M. Cole, Ph.D., M.D.

In 1949 Dr. Cole joined the Public Health Service and launched a 32 year career with NMI/NIAID. He was born in Canton, Maine in 1917. After graduating from Massachusetts State College in 1939 with a B.S., he received a Ph.D. from Harvard University in 1943, and a M.D. degree from Boston University School of Medicine in 1947. From 1943-1946, moreover, he served in the United States Army. Following his internship at Massachusetts Memorial Hospital in Boston, Dr. Cole came to NMI and was assigned to the epidemiology unit. In 1951 he became chief of the respiratory bacteriology unit within LID and was later made assistant chief. He was also head of the Bacterial Structure and Function Section, and in 1967 Chief of the Laboratory of Microbiology. In 1973 he was named Chief of the newly established Laboratory of Streptococcal Diseases. From September 1975 until December 1976 he served as Acting Scientific Director of NIAID. Dr. Cole retired from the Public Health Service in November 1981. His publications number over 100 and his research has covered aspects of sarcoidosis, herpangina, streptococci, bacterial cell wall replication, microbial ultrastructures, bacterial phages, streptococcal genetics, bacterial L-forms, and mycoplasmas. In retirement Dr. Cole has continued to pursue a life-long interest in nature photography.

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ORIGINAL ARTICLES.

HOOKWORM DISEASE (UNCINARIASIS)—A NEWLY RECOGNIZED FACTOR IN AMERICAN ANEMIAS.

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The following is an abstract of the Third Annual Address of the Brooklyn Medical Club, delivered at the Medical Society of the County of Kings Library Building, in Brooklyn, January 17, 1903. Dr. Stiles first gave a general review of the hookworms, including their history, systematic position, anatomy, and life-cycle. This was followed by a brief historical review of hookworm disease. He then gave an account of his recent trip from Washington, D. C. to Ocala, Fla., with a general summary of his observations in the Richmond penitentiary, and in the copper mines, coal mines, gold mines, brickyards, plantations, farms, orphan asylums, schools, and cotton mills of the district which he visited in the Southern Atlantic States during the course of his investigations. Turning then to the medical side of the subject he said in substance:

Relation of Soil to Anemia.—As we proceed south from Virginia to Florida we notice that anemia increases. This anemia may be divided into two general classes in particular, each class being typical of a certain kind of soil. One anemia is due to a blood infection of malarial nature, and this is found especially on the more impervious soils, as in Albany, in the southwestern part of Georgia; the other anemia is due to an intestinal infection of hookworms, belonging to the species *Uncinaria americana*, and this is preeminently a disease of the sand areas, as is seen, for instance, at Waycross, in the southeastern part of Georgia, where there are about 20 cases of hookworm disease to one case of malaria; when we reach a district with a sandy top soil and a more impervious subsoil, as at Willacoochee, Coffee County, between Albany and Waycross, we find both diseases prevalent.

The reason for this regional frequency of the diseases in question is at present clear in part only, namely: In regions of clay top or subsoil the natural breeding-places of *Anopheles* will increase in number and persist longer than in sandy regions, hence, with an initial infection of malaria the disease will spread. The relation of uncinariasis to the sand is not quite so clear, but I will endeavor to explain this point, at least in part, later in the evening.

UNCINARIASIS.

Cause.—Uncinariasis or hookworm disease is primarily a disease of warmer climates. In the Old World it is caused by a parasitic worm known as *Uncinaria duodenalis* (*Agchylostoma duodenale*), which was described by Dubini in 1843. Aside from a few, chiefly imported, cases, our American disease is caused by a totally distinct parasite, known as *Uncinaria americana*, which I described last May. The parasites live in the small intestine and suck the blood of their victims. They are in fact blood suckers of the worst type. One factor in the production of the disease is, therefore, a loss of blood to the parasite. In addition, minute hemorrhages occur from the wounds made by the worms. Further, these wounds form points of attack for bacteria. Next, the intestinal wall thickens and its digesting surface is decreased. Finally, the parasites apparently produce a poisonous substance. Thus different factors are involved in producing the symptoms noticed.

Eggs: diagnosis by fecal examination.—The eggs are laid by the female and are discharged in great numbers in the stools of the patients, a fact which gives us an excellent method of positive diagnosis, namely, a microscopic examination of the feces. Not every physician, however, owns a microscope; but I have found a substitute test which can be made by any person and in which the error is probably not over 20 or 30 per cent. in medium and severe infections. In perhaps 8 out of 10 cases of such infections, the stools are reddish brown, and if a portion is placed on a piece of white blotting paper for 20 to 60 minutes and then removed, a distinct red, blood-like stain is noticed. In this test, it is assumed that we have before us a case of anemia not suffering from piles.

Development direct.—About 24 hours after the egg is discharged in the stools, a rhabditiform embryo develops; this sheds its skin after about 48 hours; then about 5 days later it undergoes a second shedding and becomes a so-called "encysted larva"; it has now reached the infecting stage and no longer takes food until it gains access to a host, after which it sheds its skin a third and a fourth time and becomes adult.

Relation of sand to uncinariasis.—Returning

now to the relation of uncinariasis to the sand, I would suggest that when the embryo or the larva leaves the feces and enters the ground, he stands a much greater chance of gaining access to surface wells in a sandy soil than in a clay soil; again, when he sinks just below the surface he is more likely to be brought to the top again by persons walking or by children playing on the sand than on the clay, hence, chances for hand-and-mouth infection would increase; further, in a sandy soil, the worm would obtain more oxygen than in clay, and air is quite necessary to the development of these worms.

While dealing with the infecting stage, we may mention briefly the methods of infection and of prevention:

Infection.—Infection may undoubtedly take place by swallowing the worm, either in water or in contaminated food, as when a person with dirty hands eats a piece of bread; infection by biting the finger nails or by sucking the fingers, etc., when these are soiled with infested sand also calls for credence. Looss has suggested that infection may take place through the skin, and Bentley suggests that "ground-itch" is a result of cutaneous infection with the larvæ. Looss has now demonstrated the possibility of cutaneous infection, but I would mention the fact that "ground-itch" appears to exist on clay as well as on sandy soil, while uncinariasis is primarily an infection of sand areas; further, anemia can not be said to be a constant symptom after infection with ground-itch. Hence it is possible that our Southern "ground-itch" is only in part identical with the "ground-itch" studied by Bentley.

Prevention.—As for prevention, it is clear that this should consist of two methods in particular: (1) Proper disposal of feces, since infection is here present in its most concentrated form; (2) treatment of all, even light cases of uncinariasis, to decrease the chances of disseminating the infection; and to these two other points may be added, namely (3) drink pure water, and (4) keep hands and nails clean.

Symptoms of uncinariasis.—Turning now to symptoms, it may be stated that there is only one which is absolutely constant, namely, the *presence of hookworms* in the small intestine. If these worms are in the egg-laying stage, as will be found about five to six weeks after infection, *ova will be found in the feces* in proportion to the number of fertilized female parasites present in the small intestine. In light infections, we may have to examine 5 or 10 preparations carefully for half an hour or more, before finding a single egg; in heavy infections, we may find 20 to 100 eggs in the first slide.

If only two or three parasites are present, the other symptoms will be so light as to escape attention, in fact, it may be stated that the patient is practically well. As the infection increases, however, symptoms become more pronounced, until we reach the extreme stage represented by the so-called "dirt-eater." We have now a train of symptoms more common on a sandy than on a clay soil; more common in rural districts than in cities and towns; more severe among women and children than among men over 26 years old; apparently more severe, or at least more noticeable in blonds than in brunettes; more severe among whites than among negroes; like trichinosis, several cases usually occur in the same family, and, as a rule, also among neighboring families; above the frost-line the symptoms increase from spring to fall, and decrease from fall to spring.

Anemia is one of the most common symptoms, and is usually erroneously attributed to malaria and poor food. Its degree varies, of course, according to the stage and the degree of infection. The *skin* varies from a waxy white to a tallow or a parchment-like yellow to tan. In some cases, the visible *mucous membranes* still retain their reddish tinge, in others they become a marble white. The *cardiac symptoms* are of course prominent, and many of the patients speak of their trouble as "heart disease"; the cervical pulsations, or "neck jerking" in the sand-hill vernacular, may be seen at a distance of 6 to 12 feet; the *pulse* ranges from 80 to 132, without any correspondingly high *temperature;* the latter may be subnormal or normal, although 100 to 102° F. is not rare.

With the anemia we may have an irregularly appearing *edema*, known among the patients as "bloat," "swelling," or "puffiness"; the face and ankles are the parts most frequently affected. The term "bloat" is also applied to the swollen abdomen, which is very common; this is usually referred to as "pot-belly" or "buttermilk belly," and often develops to such an extent that nearly all the men, women, and children of a given neighborhood look as if they were about 6 months' pregnant.

With the anemia, there is usually also a *progressive emaciation*, so that the severest cases appear like living pregnant skeletons. The *muscles* are soft and flabby, and naturally the patients are weak and unable to work. Partially connected with the muscular weakness, I may also mention

the *difficulty in breathing*. One of the most striking symptoms seen in infections of long standing is the *stunted growth*, both physical and mental. A boy or girl of 14 to 18 may appear to be only 10 to 14 years of age, and I have seen men and women 20 to 22 years old who were not better developed than a child of 12. These patients, small in stature, have little or no hair on their body; the penis may be as small as the end joint of the little finger; in a boy of 17, the testicles have not always descended; in a girl of 16 to 22 the breast may be absolutely undeveloped, the vulva not larger than that of a child of 11 or 12, and the patient has perhaps never menstruated or does not menstruate over two or three times a year, then chiefly in the winter months.

I was unable to confirm the view that *sterility* is very common in uncinariasis, but I did find that *miscarriage* was exceedingly common among infected families. Whether these miscarriages were actually due to uncinariasis or to some other cause, must be left an open question. In some cases there was a clear history of venereal disease; in others, it occurred after severe work in the field; in others, the women thought they had malaria and had taken large doses of quinine.

These women age very fast. I have seen a woman of 26, with three children, who looked to be 50 and was totally broken down; and I have seen a woman of 40, with 10 children, who looked to be 70; one woman of about 48, with a history of 18 children and 2 abortions, was a physical wreck.

Parents and teachers complain that the infected children are *backward in their studies*, and the children complain that to study brings on severe *headaches*.

The appetite and bowels are irregular. There may be *diarrhea or constipation*. The *appetite* may be light to ravenous, and it very frequently develops in some special *abnormal* direction: one patient will be especially fond of strong coffee, without sugar or milk; another chews coffee most of the time; another eats salt or sucks lemons, or lemons and salt; another is noted for miles around on account of his appetite for pickles; another eats wood; another chews rags; some eat plaster; others eat sand; still others eat clay; and I have found a record of a negro who ate live mice! This abnormal appetite is, I believe, a result of the intestinal irritation and the anemia, and corresponds exactly to what we find in dogs, cattle, sheep, goats, seals, and elephants infested with intestinal worms.

Several recently published articles have referred to peculiar *markings on the tongue* in cases of uncinariasis, and this symptom has been recorded in early writings on dirt eating. It certainly was more or less common in the patients I saw, but in a few cases I suspected that it was possibly due more or less to tobacco or snuff.

One very constant symptom which I found was a peculiar cadaveric or *fish-like stare* to the eye. If a patient is directed to stare intently at the observer, the pupil is seen to be dilated or to dilate and the eye assumes a blank, stupid, cadaveric stare, quite similar to the stare noted in persons in extreme alcoholic intoxication or to the stare of a fish's eye. In only two cases where I noted this symptom, did I fail to find the eggs when the stools were examined; those two cases, 12 and 14 years old, respectively, were sons of a confirmed drunkard, and one of them showed infection with whipworms (*Trichuris trichiura*). This peculiar symptom was not noticed, however, in any of the severe cases of malaria which came under my observation.

Lethality of uncinariasis.—The exact lethality of American uncinariasis must be left undetermined for the present. Severe infections are undoubtedly serious and often fatal, but from the fact that such a large proportion of children are infected in a given locality, and some of these seriously, I obtained the general impression that uncinariasis *per se* is not quite so fatal as one would naturally expect. But let a patient who is seriously infected with this disease contract typhoid, tuberculosis, pneumonia, or severe malaria, and the second illness will be of comparatively short duration. While my observations have not yet extended over a long enough period to speak with certainty upon the subject, I am not disinclined to the belief that we shall eventually all unite in the view that many of the deaths attributed to uncinariasis in man are in reality due to a second disease which the patient was not able to withstand because of the preëxisting hookworm infection. Further, it is evident that with the disappearance of uncinariasis from the sand districts, the proportion of deaths from tuberculosis, typhoid, pneumonia, malaria, childbirth, etc., must necessarily decrease.

Frequency of uncinariasis.—I have already stated that locally infected cases of uncinariasis in cities, towns, and in clay districts, are not frequent. In cities and towns the conditions are unfavorable to the development of local foci of infection, because the streets and walks are paved, yards are sodded, and sewerage is present. As

we approach the outskirts of the city and enter the country, paved streets and walks decrease or disappear; yards are not sodded; and the sewerage systems are supplanted by box privies. Thus, conditions become more favorable for uncinariasis and we find that this increases in sandy regions as we leave the city and in proportion to the attention given to the care of the construction and cleaning of the outhouses. Cases of uncinariasis found in cities and towns have usually come in from the country, hence, when estimating the frequency of this disease we must practically eliminate the city population from the possibility of local infection and recall that an anemia within the city limits, as well as an anemia in rural clay or stone districts, is much more likely to be due to malaria than it is to uncinariasis. By this, I do not, of course, mean to advance the view that malaria is preëminently a disease of cities, but simply to call attention to the fact that cities present conditions which are more favorable to the development of malaria than of uncinariasis.

Holding these facts in mind, I should estimate that in the strictly sand districts, uncinariasis is the most common disease of the white population of the South. In the negro it is less severe, and perhaps less common, than tuberculosis, syphilis, or gonorrhea. Taking the South as a whole, I should say at present that uncinariasis should be placed in the same general category as malaria, tuberculosis, syphilis, and gonorrhea.

Economic importance.—From an economic standpoint, uncinariasis must rival and probably exceed malaria in importance. Its presence prevents the proper education of the children, prevents the proper development of the youth, and decreases the labor-producing power of the adult, hence, of the productiveness of the farm. It increases the number of orphans, and the number of people who must be supported by charity. It increases expenses for drugs and medical attendance.

As illustrations of these points, I have seen families of 12, all of whom were affected with uncinariasis; in one family of 22 members, there had been 11 deaths, including 2 miscarried fetuses; on one farm of 60 hands, 20 were examined and all found infected; the hands of that farm were not producing over 70 to 80 per cent. of normal labor, and in one family I saw on another farm, the labor did not exceed 30 per cent. of normal work. In three orphan asylums, I found about 8 to 15 per cent. of the children infected with uncinariasis.

Uncinariasis in the cotton mills.—I visited three cotton mills, which I was assured presented average typical conditions for such institutions, and one mill which I was assured was far above the average. Taking the first three as basis for my remarks, it may be said that the amount of anemia and the number of small children among the hands were very striking. Several children were pointed out as the typical "mill children," of whom we have heard so much. It took but a glance to recognize that they presented the type of stunted growth so common in uncinariasis; inquiry developed the fact that they had come from the rural sand districts, and physical examination gave a clear diagnosis of hookworm disease. Visiting a number of mill families in company with the contract physician, it was not difficult to prove that uncinariasis was more or less common, but that with continued residence in the city, the infected cases improved, unless they were too far gone to admit of recovery. The effects of former infection were clearly discernible in two mill men who had been in the city, they claimed, 13 years.

It is not my intention to account for all the anemia and other conditions of the mills by attributing them to uncinariasis, for other factors, such as malaria, the frightful prevalence of tobacco chewing and snuff dipping among even the youngest hands, the constant breathing in of fine particles of cotton, etc., come into consideration, but I would submit that uncinariasis represents a new factor in the subject of the child labor of the cotton mills. While it is not a complete solution of the problems involved, it would be well for us to recall that these children from the sand areas are from one to five years older than they appear; at the mills they are earning more money, have lighter work, are living in more hygienic houses, are in better health and better spirits, have more regular medical attendance and better chances for recovery than these same people had on the sand farms. I do not mean to picture them as giants of strength or their houses as palaces, but to properly judge them we must compare the conditions at the mills with the conditions on the farms, and the mill hands with the farm hands.

General effects on the inhabitants as a class.—We are all familiar with the general characteristics attributed to the poorer white population in certain parts of the South, namely, that class so frequently referred to under the not very rhetorical expression of "the poor white trash." To fully appreciate just what these people are and how they live, we must see them in their homes. Now it has been my experience that these people

present a more typical picture in the sand regions than in clay localities. The poorer whites in the clay regions and in the cities are healthier and more active than those in sand districts. Let a family move from sand to clay, and it improves; let it move from clay to sand and it runs down. This idea that a clay soil is healthier than a sandy soil is common knowledge among many of these people. With the discovery of the prevalence of uncinariasis in the sand localities, we have an explanation of these facts which, though it sounds extreme and sensational, is one which demands serious attention. Personally, after a careful study of eight weeks in the localities in question, and after seeing numerous cases of uncinariasis, I see no possible escape from the conclusion that this disease offers us a pathologic basis for the facts mentioned, and that this is one of the most important, if not the most important, factor in the inferior mental and physical development of these people. Eradicate uncinariasis and these poorer whites will, in one or two generations, be a different people; or let the conditions remain as they are, and any whites who go to the sand farms and live as these people do, will deteriorate to the level of the so-called "dirt eaters" and "poor white trash."

How to change the present conditions.—To alter present conditions, the first thing *not* to do is to try to teach the people to boil their water and keep the hands of their children clean. The proposition of boiling or filtering drinking water is absurd to the average mind of the laboring classes; and the average mother of three to ten children on a "one-horse farm" has something else to do besides washing the hands of her children every time they eat. Any campaign in this matter which we adopt must be practical, not academic. By pursuing an academic course, we shall lose all our influence with the average uneducated man.

The first point is to disseminate widespread among the country physicians an account of the cause, symptoms, cure, and prevention of uncinariasis. This should not be obscured by technical words which not half of the physicians of the world understand.

The next point is to educate the rural population to a more general and a better construction and care of privies, which should be located in such a place that the drinking water can not be infected. This can best be done by the family physician, who has the confidence of the people. Efforts by strangers will meet with suspicion, but the family physician can present to the family the filth connected with the water contamination and the financial side of the question in the loss of income, expense for drugs, etc. One practical demonstration in a neighborhood will do more than any amount of agitation. Hand in hand with these measures of prevention, one of the medium severe cases in the family should be treated as a practical object lesson. After the recovery of this patient, the family will be only too anxious to follow out directions, and with an improvement in the general condition of the family, the cleanliness will more or less take care of itself, especially if the children go to school. I doubt, however, whether the average farm hand will ever come to the point of boiling or filtering the drinking water.

Treatment.—The method of treatment is very simple, but contains one prominent pitfall, namely, the impression that a single dose of medicine can be administered and the patient discharged as cured. As a rule, large doses of thymol or large doses of male fern are given. If the former, the physician should absolutely prohibit the use of alcohol during the day of treatment. One method is as follows:

8 A. M. 2 grams (31 grains) *powdered* thymol in capsules.

10 A. M. Repeat.

12 Noon. Dose of salts or of castor oil.

Repeat treatment once a week until stools show no evidence of eggs.

If male fern is used, comparatively high (and sometimes fatal) doses are usually given, namely, from 10.0 (2½ fluidrams) to 20.0 (5 fluidrams).

Uncinariasis in Northern Practice.—With the possible exceptions of mining districts, probably most of the cases of hookworm disease observed by Northern practitioners will be imported from Europe, the Philippines, the Southern States, Cuba, Porto Rico, Central and South America. If from Europe, Asia, Africa, or the Philippines, *Agchylostoma duodenale* will be usually found. If from the American continent, *Uncinaria americana* is more likely to be found. To the Northern physician, who has the patient isolated from the source of infection, it is, of course, of importance to determine how long the parasites will persist in the intestine. Bearing upon this point I can say that I have one case, which is free from criticism, where *Uncinaria americana* has lived 6 years and 7 months; two cases, likewise free from criticism, where the infection has persisted 6 years; finally, one case, not free from criticism, where the infection *may have* existed 10 years.

It is needless for me to insist upon the practi-

cal application of this point. Here in Brooklyn you may have a case of obscure anemia; in such a case it is necessary to go back for 10 years in the history to see whether the patient has during that time visited any sand areas in the Southern States or elsewhere in warmer climates.

INFANT DIARRHEAL MORTALITY IN BROOKLYN. ITS CAUSE AND PREVENTABILITY.

BY LOUIS C. AGER, M.D.

Read at the Meeting of the Kings County Medical Association, December 6, 1902.

The title of this paper properly embraces two questions:

I. The cause and preventability of the high summer infant mortality in all large cities.

II. The cause and preventability of the excess of Brooklyn's summer infant mortality over that of Manhattan.

The paper will be mainly devoted to the second question, but most of the deduction and suggestions will apply equally to other large cities.

The statistics are derived from three sources:

I. The U. S. Census of 1900.

II. Various reports of the New York and Brooklyn Health Departments.

III. Personal investigation of 250 fatal cases of diarrheal disease in Brooklyn and 519 cases in Manhattan.

The "exciting cause" of this paper may be concisely shown in Tables I. and II. Table II. particularly shows the marked difference in the diarrheal mortality in Brooklyn and Manhattan,—a difference of from 30 to 40 per 100,000 of population on the wrong side of the sheet for the credit of Brooklyn. The figures in Table II. are estimated on the new classification of causes of deaths and cannot be compared with the figures in Table I. Under the old classification, "Diarrheal Diseases" included diarrhea, dysentery, cholera morbus and entero-colitis, while enteritis and gastro-enteritis were classed under "Digestive Diseases." Now the two latter have been added to the diarrheal class. Table I. has been prepared to show how long the rate has been higher in Brooklyn than in Manhattan. From 1881 to 1891 inclusive Manhattan had a higher rate than Brooklyn, with the exception of two years. Since 1891, with the exception of two years, Brooklyn has had the higher rate.

The first fact that attracts attention in Table I. is that in twenty years the diarrheal death rate has been more than cut in half. But the next thought is that during the second decade New York has far outstripped Brooklyn in improvement. Two possibilities suggest themselves to invalidate these comparisons. The first, a possible difference in methods of classification, might have had some weight ten years ago, but since consolidation the systems have been identical. The second possibility was that there might be a difference in child population. Unfortunately the United States Census for 1900, Table III., shows that the difference is in favor of Manhattan. This fact is, I believe, contrary to the general belief in the matter.

This leaves unexplained the fact that Brooklyn, with its long standing reputation for excellent sanitation and low death rate, has a higher infant mortality than Manhattan, which contains some of the most densely populated areas in the world.

Tables IV. and V. show that not only Brooklyn makes a poor showing, but that the other boroughs are even more in need of investigation. In fact it seems to be the rule in Greater New York that the diarrheal mortality is inversely as the density of population. Differentiating still further, Table VI. shows that the three suburban wards of the Borough of Brooklyn with no tenement population and with a different water supply, had during July, 1902, almost the same infant death rate as the rest of the borough.

It is the fashion at present to look upon "poor milk" as the root of all diarrheal evils, but improper diet is a better statement of the case. All who have spent their summers in country districts are aware that it is easier to get clean, rich, sweet-flavored milk here in Brooklyn than on a farm. Moreover, there is practically no difference between the milk in Brooklyn and that in Manhattan.

The Brooklyn water supply also comes in for a share of the blame, but two facts weaken this argument. First, the high death rate prevails in the wards not supplied with Ridgewood water. Second, the rate is even higher in the other boroughs.

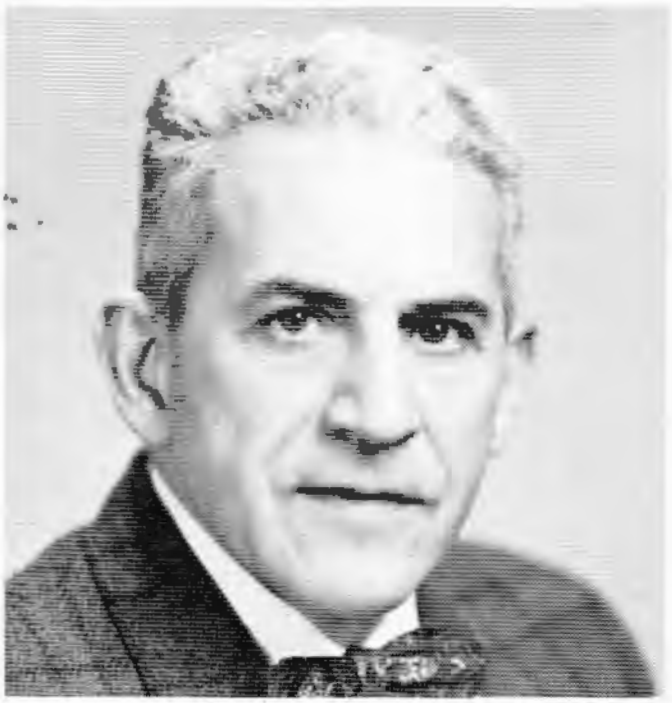
Several facts from widely different sources are very suggestive when brought together in this connection. First, certain English observers have shown that when subsoil temperature reaches about 56° F. there is a rapid increase in diarrheal diseases. They therefore suggest that the specific organisms are found in the soil and that they develop rapidly with a favorable temperature. Second, the claim of Duval and Bassett, in Baltimore, to have demonstrated that the Bacillus Dysenteriæ of Shiga is the specific organism in diar-

Significant Contributions from the Division of Zoology and Its Successor Laboratories at the NIH, 1902-1970.

Leon Jacobs

The history of medical parasitology at the Hygienic Laboratory starts with Charles Wardell Stiles. As a student at the University of Leipzig and other institutions, Stiles acquired from a number of distinguished German investigators, in addition to enormous amounts of information, the attitude of the "Geheimrat." He returned to the United States and took a position in the Bureau of Animal Industry of the U.S. Department of Agriculture (USDA). A prolific worker, Stiles published a considerable amount of information on parasites of livestock. His attention was drawn to hookworm by Bailey K. Ashford, a former student working in Puerto Rico, and by Thomas A. Claytor, a Texas physician who described the first clinical case of human hookworm disease in the United States. Stiles identified the parasite, naming the new species of hookworm *Uncinaria americana*, now known as *Necator americanus*, which is an important parasite of human beings.[1] This information came to the attention of Milton J. Rosenau, Director of the Hygienic Laboratory, and Surgeon General Walter W. Wyman of the U.S. Public Health and Marine Hospital Service, precursor of the U.S. Public Health Service (PHS). Rosenau and Wyman recruited Stiles to initiate programs in medical zoology within the Hygienic Laboratory. Stiles must also be credited with interesting John D. Rockefeller, through the efforts of Walter Hines Page, in the problem of hookworm in the South, an interest which led eventually to the establishment of the Rockefeller Foundation. The original Rockefeller campaign against hookworm was also an impetus for the development of strong parasitology programs at the Johns Hopkins School of Hygiene and Public Health and elsewhere.

Stiles's interests in parasites were broad. He published extensively on helminths and also recorded the first case of giardiasis in the United States. However, towards the end of his career he spent most of his energy recording the literature of parasitology in the Index Catalog of Medical and Veterinary Zoology, which is still extant. After Stiles's retirement, George W. McCoy, director of the then singular National Institute of Health, again turned to the USDA Bureau of Animal Industry to find a new leader for the program in medical zoology. In 1936 Maurice C. Hall, a veterinarian with a Ph.D. in zoology, was appointed as Chief of the Division of Zoology. He brought with him from the USDA a group of investigators who had already made significant contributions in veterinary parasitology, including Willard H. Wright, Eloise B. Cram, Myrna F. Jones, and John



Leon Jacobs

Bozicevich. Hall outlined a program for this group, based on his perception of parasitic diseases which continued to surmount our sanitary barriers. These were trichinosis, oxyuriasis, and amebiasis. After Hall's sudden death in 1938, Wright was named to direct the Division of Zoology. Wright was also both a veterinarian and Ph.D. in zoology. With imagination and considerable ability he continued the earlier programs and added new ones. The Division of Zoology incorporated malaria programs conducted elsewhere and eventually evolved with the incorporation of arbovirology, into the Laboratory of Tropical Diseases. From 1938 until his retirement in 1958 the Laboratory of Tropical Diseases was headed by Wright.

It is well to pause here and make note of the fact that in the early days the Hygienic Laboratory and the National Institute of Health were oriented not only to research but also to the prevention and control of disease. Thus, some of the early studies on the prevalence of trichinosis in the United States, first done by Stiles and then continued and extended systematically by Hall and Wright, outlined the importance of disease as a public health problem and defined the characteristics

of the nematode parasite and the methods for its control. Indeed, it was not until after World War II that a delineation of functions, between research and control, began to develop in the Public Health Service. This divergence resulted in the creation of the Communicable Disease Center, now the Centers for Disease Control (CDC). The CDC has acquired its own highly respected reputation for excellence, but areas of cooperation between the two institutions remain. It is important, after these parenthetical remarks, to make clear that some of the early studies done at NIH were motivated by its larger mission as the principal PHS research and control institution. This explains some of the more practical papers that will be cited henceforth.

It is also important to note that specific research goals were established and pursued by some of our early leaders. Whether an individual research project was basic or applied mattered little, so long as it fitted into the overall objectives. This was true, indeed, of most biomedical research at that time. The purpose of research was to accrue more and more information that would lead to the conquest of disease. The individual paper was a contribution to that end. Each paper represented another building block in the necessary structure of the science. In the words of the distinguished Rockefeller Foundation malariologist, Lewis W. Hackett, who served for some years as editor of the *American Journal of Tropical Medicine and Hygiene*, "We build like coral polyps." The exposition which follows is based on this idea: that a number of contributions have to be cited to reveal the development of major landmarks.

Maurice C. Hall and his colleagues instituted a series of studies of trichinosis after Hall came to NIH from the USDA Bureau of Animal Industry. These studies first delineated the problem of the extent of infection and disease due to *Trichinella spiralis*,[2,3] and some studies were done on the control of infection.[4,5] Also, Bozicevich and his colleagues did some of the early work on the immunodiagnosis of parasitic infections[6] and introduced the bentonite flocculation test for trichinosis which was widely used for many years.[7] In the course of some of the studies of immune reactions in trichinosis, Bozicevich and Detre made one of the earliest demonstrations of the occurrence of circulating antigens in recently infected animals.[8] This was a precursor to later work on the occurrence of antigens and antigen-antibody complexes in a variety of infections and disease states.

During World War II, Wright directed some of the efforts of the Division of Zoology to appraising the risks of importation of vector-borne parasitic diseases to the United States in returning troops. Studies were conducted on potential mosquito vectors of filariid nematodes,[9] and similar work was done on North American snails as possible vectors of schistosomiasis.[10,11,12] The work on filariid vectors was considered especially important, because a focus of endemic filariasis had previously existed in Charleston, South Carolina, having probably arrived originally from Africa. It was not to be considered unlikely that other strains of the same filariid from other parts of the world, or different filariids, could find



Dr. Jacobs, second row, far right, with Division of Zoology in 1938.

suitable mosquito vectors here. The most significant work on schistosomiasis vectors was the later demonstration by Newton of the role of genetics in observed strain differences in susceptibility and other host-parasite interactions of snail vectors.[13,14] This genetics work was later expanded by Richards to show characteristics of different strains of snail, and also genetic differences in the infectivity of schistosomes to strains of snails.[15]

Other work done in the Division of Zoology and Laboratory of Tropical Diseases involved the efficacy of sewage- and water-treatment in the control of parasitic infections. Basic work on the effects of a number of agents and treatments on parasite eggs and cysts was contributed.[16,17,18] Some of the early data were important in military operations during World War II for bivouacking in the field.

Enterobiasis was another early focus identified by Hall for the Division of Zoology. Its pathogenesis as a pediatric infection had not been established, and this depended on a reliable diagnostic technique, because the eggs of the worm are not passed in feces. The technique developed at the NIH relies on the demonstration of eggs on the skin of the perineal region, where they are deposited by the female. Developed by Hall, it is known as the "NIH swab" and is still the accepted technique.[19] Other work done on the pinworm involved the incidence and epidemiology of the infection, including such factors as the viability of the pinworm egg, and on treatment.[20,21,22] In connection with attempts at control early work on the effects of radiation on living organisms was done in the Division of Zoology with Alexander Hollaender who was in physical biology. This involved tests of monochromatic ultraviolet radiation on the pinworm eggs.[23,24]

Additional important work was done after World War II, when radioisotopes first became available. Frederick J. Brady collaborated with Dean Cowie of the Carnegie Institute of Terrestrial Magnetism and others on some of the first uses of radioisotopes in pharmacology.[25] This involved synthesizing drugs with radioactive elements to identify the sites of activity of such drugs in animals infected with filariid parasites.

A considerable problem in studying amebiasis and amebic dysentery before 1961 was the inability of investigators to grow pure cultures of the amoeba, *Entamoeba histolytica*, or any of its close relatives. Cultures were always contaminated with bacteria and could not be maintained without bacteria. Starting about 1940, a series of studies at NIH resulted in the development of cultures of the amoeba with single species of bacteria, by the technique of micro-isolating cysts of the parasite, washing them repetitively in sterile water, and inoculating them into cultures with selected bacteria.[26] Later, when antibiotics became available, it was possible to eliminate bacteria from cultures and to maintain them briefly without bacteria,[27] or to substitute other bacteria, or protozoa[28] instead of bacteria. Finally, cultures of the amoeba without any other organisms were obtained and maintained indefinitely. Thus, a whole series of studies eventually culminated in a great landmark.[29] Similar techniques led to the cultivation of other species of parasitic protozoa in axenic cultures,[30,31] which subsequently allowed studies on the biochemistry of the organisms.[32,33]

Physiology of parasites was the principal effort of one distinguished scientist recruited by Wright. Displaced by the Nazis before World War II, Theodor von Brand made major contributions in describing the aerobic fermentation of blood trypanosomes and the anaerobic metabolism of parasites. It is difficult to single out individual papers as his most significant contributions, because his work and that of contemporaries in the same field is voluminous and very detailed. Many of his truly remarkable contributions, however, are summarized in two books.[34,35]

In malaria research, the volume of work done by members of the staff of the Laboratory of Parasitic Diseases and its antecedent organizational components was likewise large and important. A group of investigators in South Carolina, exploiting the then-current clinical use of induced malaria to control syphilitic dementias, studied the characteristics of strains of malaria obtained from many different parts of the world to elicit information on the relapse patterns of *Plasmodium vivax* strains.[36] They provided information on other species of malaria[37] of considerable importance regarding the occurrence of dormant forms of the malaria parasite, and this information contributed to the eventual description, in 1948, by Short and Garnham, of exoerythrocytic stages of *P. vivax*. Citations of some of these early studies are found in the symposium already cited,[36] on human malaria published by the American Association for the Advancement of Science in 1941. A great deal of the work on malaria during the years of World War II was a coordinated effort on drug-development, headed by James A. Shannon, who was to become, years later, the Director of NIH. A record of the findings on almost 4000 substances studied in a chicken malaria system at NIH from 1941 until 1952, exists in a Public Health Service Publication, No. 193, which appeared concurrently with *Public Health Reports*, v. 68, no. 1, in January 1953.[38]

Early work on malaria involved careful and accurate identification of the stages of the parasite in its natural hosts in the field and in experimental hosts in the laboratory. In 1942, in recognition of the need for identification of the parasites' stages, the NIH sponsored the publication of a manual describing them. This manual was prepared by Aimee Wilcox, with the assistance of the NIH resident artist, Inez Demonet. Its color plates of the morphology of the malaria parasites have been the standard for years and have been reproduced many times for different texts.[39] Contributions to the study of candidate antimalarial agents in man were also made by NIH researchers. One example is a short paper describing the method by which a great deal of basic information on drugs and on relapse patterns of the malarias was obtained in the period from World War II through the 1970's.[40]

In addition to drug-screening techniques and clear cut clinical studies, field work on malaria was also carried out by members of the Laboratory. In the course of such work in Malaya, one of the staff discovered the transmission of a simian malaria parasite to man, by vectors that were colonized in the laboratory. The principal investigator, Don Eyles, suffered himself from the infection, due to *Plasmodium cynomolgi bastianelli*.[41] Another facet of malaria pharmacology involved the resistance of the parasites to drugs. The first rigorously documented study of resistance of *Plasmodium falciparum* to the drug chloroquine was provided by NIH investigators, after the drug had been in use for fifteen years.[42]

Other progress in malaria research involved collaboration of malaria specialists with individuals interested in the immunology of parasitic infections. One major contribution was the demonstration that the technique of immunofluorescence could be applied to the diagnosis of malaria infections.[43,44] The later development of efforts towards malaria vaccines derives from the original observations on antigenicity of the parasites. In addition, research on malaria therapy contributed to the treatment of at least one other disease. The drug, pyrimethamine (Daraprim) had been shown in malaria studies to have considerable usefulness. One of the malaria investigators, Joseph Greenberg, suggested to Eyles that it be tried against *Toxoplasma gondii*. Eyles was then undertaking chemotherapeutic trials against *T. gondii* in a Memphis, Tennessee laboratory field station of the Laboratory of Tropical Diseases. Eyles and Coleman[45] not only demonstrated the efficacy of pyrimethamine against *Toxoplasma* but very neatly showed that the drug was synergistic with sulfa drugs. This paper was the first to outline a treatment for toxoplasmosis and the drugs still remain the most accepted treatment.

In the field of helminthic infections, some additional

mention should be made of the filarial parasites. Work on testing the potential of domestic mosquitoes to serve as vectors of *Wuchereria* has already been described. In other work, LPD investigators studying *Dirofilaria* infections in dogs as models of human disease found that, in addition to *D. immitis*, another dog filariid occurs in the U. S., and that this parasite is less pathogenic to the dog.[46,47] This finding has considerable significance in veterinary medicine, because the heartworm (*D. immitis*) causes a great deal of morbidity and mortality while the other filariid poses no severe clinical problems. In addition, this discovery demonstrates how disease studies in animal models contribute materially to our knowledge of animal diseases (a point to make to anti-vivisectionists, even though it is hardly likely that they will listen).

Research on onchocerciasis in Guatemala was performed by LPD scientists working in collaboration with the Pan American Health Organization (formerly the Pan American Sanitary Bureau). The most significant result of this work was a monograph on the blackfly vectors of the filariid nematode, *Onchocerca volvulus*. In addition to the description of species, this work also provided vital information on the breeding places of the flies, on their flight ranges (which are very long), and on their vector potential.[48] Other studies on filariasis, pursued by LPD scientists in the South Pacific, resulted in significant contributions to our understanding of the bionomics of the vectors of human aperiodic filariasis. Another important discovery, however, derived from the presence of one of our scientists in that locale. Leon Rosen identified a clinical disease there called eosinophilic meningitis and was able later to recover a nematode worm from the brain of a fatal case of the disease. It was identified as a larval form of a worm ordinarily found as an adult in the rat. Rosen and colleagues also worked out the epidemiology of the human disease, which involves invertebrate animals capable of serving as intermediate hosts of the nematode.[49]

Additional work on helminths involved the pathogenesis of human schistosomes and some of their avian relatives. In early work, Olivier was able to show that the phenomenon of schistosome dermatitis was due to sensitization to larvae of avian schistosomes that were attempting to invade human skin. This was the first well-controlled study of the phenomenon, demonstrating that lesions increase with repeated exposure.[50] Similarly, Cheever found, in the first careful quantitative study of human schistosomiasis, that lesions increased in size as well as number with increase in worm burden.[51] Weinbach, working on the use of the pentachlorophenols as molluscicides for the control of schistosomiasis, demonstrated that the mechanism of action of these compounds is the uncoupling of oxidative phosphorylation in mitochondria. He and a colleague later demonstrated that rates of oxidative phosphorylation in mitochondria varied with the age of the animal from which the enzymes were derived.[52,53]

In 1956 Weinstein and Jones contributed landmark research on helminths when they cultivated larvae of a nematode parasite to the adult stage *in vitro*.[54] This provided the basis for cultivation of a number of other helminths later and for significant contributions to the physiology of these organisms.[55]

Jacobs and colleagues contributed information on the biology of the intracellular parasite, *Toxoplasma gondii*, on the resistance of the cysts of this parasite,[56] and on the occurrence of the cysts in the flesh of meat animals[57]—all of considerable significance in the epidemiology of the infection. They developed the hemagglutination test for toxoplasmosis as a substitute for the difficult and dangerous dye test which was then the only accepted diagnostic procedure.[58] They also demonstrated the occurrence of *T. gondii* in the eye of adult human beings, and through the interpretation of low serological titers, were able to demonstrate the significance of toxoplasmosis as a cause of ocular disease.[59,60] Later, Sheffield and Melton contributed to the elucidation of the life cycle and morphology of *Toxoplasma*, providing convincing evidence of the coccidian nature of the parasite and of the existence of an intestinal stage in the cat.

Conclusion

The aim of this essay has been to present an overview of the evolution of studies in medical zoology throughout the early years of the Hygienic Laboratory and NIH. Although other authors might have chosen different papers, the selections here were made according to the larger context of developments in basic or applied research or in the epidemiology of disease. These papers are, indeed, the individual coral skeletons to which Lewis Hackett referred--together they comprise a body of knowledge. In some instances, the citations are given principally to indicate the varied directions in which the work of the zoology laboratories went. (Members of the Laboratory of Parasitic Diseases could undoubtedly question the choice of citations, but the goal was merely to indicate the type of research.) In other instances, as has been pointed out, the citations represent highly significant advances of which we are all proud. It may be of interest to note that when the editor of the *Journal of Parasitology* solicited from the Institute for Scientific Information (ISI) a listing of the ten papers in the *Journal of Parasitology* that were most cited during the period of 1961 through 1982, seven of the ten papers identified were from the NIH. All of these were published before or in 1970 (*Journal of Parasitology* 69(4): 774). It should, of course, be noted that this essay has focused solely on contributions from the laboratories at NIH and of necessity has neglected other sources. As this review ends with 1970, the many new and technically sophisticated methods used today in the study of parasites and the diseases they cause, are reflected in the annotated bibliographies prepared by the current laboratory chiefs, which indicate that medical parasitology continues to expand in scope and depth.

Leon Jacobs, Ph.D.

Dr. Leon Jacobs worked for 42 years at NIH both as a bench scientist and as an administrator. Born in Brooklyn, New York in 1915, he graduated from Brooklyn College in 1935, and two years later, joined the Division of Zoology. After serving in the Army from 1943-46, principally as a Malaria Control Officer in the South Atlantic Theater of Operations, he obtained a Ph.D. in 1947 from George Washington University. From 1946 to 1966 he held various positions in NIH/NMI/NIAID. In 1959 he became Chief of the Laboratory of Parasitic Diseases and in 1964-65, he was named Acting Scientific Director of NIAID. Dr. Jacobs left NIH in 1967 to serve as Deputy Assistant Secretary for Science, DHEW. Returning to NIH in 1969 he was Assistant Director for Collaborative Research. From 1972 to 1978 he was Associate Director for Collaborative Research. In July 1978 he was appointed Director of the Fogarty International Center. Dr. Jacobs retired from Federal service in 1979. He has published over 100 papers, and edited several journals including the *Journal of Parasitology*. As Scientist Emeritus at NIH, he has an ongoing research project on toxoplasmosis at LPD and retains an active interest in studying amebiasis and other parasitic diseases.

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used by nose and throat men for their reflected light. The detection of albumin is most satisfactorily done in connection with the heat and acid method. After heating and adding acid to the specimen the test-tube containing the fluid is held directly in front of the bull's-eye lantern and the specimen viewed from a point at right angles to the rays of light. In specimens in which I and others have failed to detect any cloudiness in all other lights, the cloudiness has stood out plainly when submitted to the above procedure. The urine should always be filtered, especially for the contact test. Any bull's-eye lantern will suffice. The great advantage of this method lies in the fact that it can be done at any time—night or day.

ABORTIVE CASES OF POLIOMYELITIS

AN EXPERIMENTAL DEMONSTRATION OF SPECIFIC IMMUNE BODIES IN THEIR BLOOD-SERUM *

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It has been pretty generally recognized, since the publication of Wickman's studies on epidemic acute anterior poliomyelitis in Sweden in 1905-06,[1] that cases of this disease may run their course without resultant paralysis. The evidence on which the occurrence of such so-called "abortive" cases is predicated is, briefly, the following:

Especially in epidemics, cases of acute anterior poliomyelitis are encountered showing all gradations in the degree and extent of paralysis. In the same group may be found cases resulting in extensive and lasting paralysis; cases with permanent paralysis of slight extent; cases in which the patients have transient paralysis, recovering completely within a few weeks or even a few days; other cases in which there is no definite paralysis, but merely muscular weakness, of short duration; still others in which the only motor disturbance is ataxia, tremor or a transient ocular disturbance such as diplopia or nystagmus. Finally, within the same group are seen cases of illness exhibiting only the symptoms of a general infection, usually accompanied by symptoms indicative of meningeal, spinal or encephalitic irritation, but without definite motor disturbances.

The diagnosis of acute anterior poliomyelitis in these cases is suggested usually by their close association with typical cases of the disease rather than by the distinctiveness of their symptoms. The symptoms are, however, sufficiently similar to the pre-paralytic symptoms observed in paralytic cases, and in many instances sufficiently different from the symptoms observed in the more common infectious diseases to justify a provisional diagnosis of poliomyelitis. The similarity of these abortive cases to cases of frank poliomyelitis in the early stage, the signs of involvement of the nervous system, their close and constant association with frank paralytic cases, and the gradation of symptoms above mentioned had convinced many observers of the etiologic identity of abortive and paralytic cases of poliomyelitis even before any light had been thrown on the subject by the study of experimental poliomyelitis.

It has recently been noted by several observers that monkeys inoculated with the virus of poliomyelitis occasionally pass through an indefinite illness without resultant paralysis, clinically similar to abortive attacks as observed in man.

It has been shown by Levaditi and Landsteiner,[2] Romer and Joseph[3] and Flexner and Lewis[4] that the serum of monkeys which have recovered from poliomyelitis, mixed in suitable proportions with an emulsion of the virus and allowed to remain in contact for a sufficient length of time, renders the virus inactive, so that when injected into fresh monkeys it fails to produce the disease.

Netter and Levaditi,[5] and Flexner and Lewis,[4] have demonstrated the same property in the serum of human beings who have recovered from acute poliomyelitis. This property has been found by all the above observers to be absent from the serum of normal persons and monkeys, and has therefore been considered a specific property, the demonstration of which in the serum justifies the inference that the subject has passed through the infection of acute anterior poliomyelitis.

Netter and Levaditi,[6] availing themselves of this test, have also demonstrated this germicidal property against the virus of poliomyelitis in the serum of a suspected abortive case, thereby giving additional confirmation of the etiologic identity of such cases with frank poliomyelitis.

The clinical history of the abortive case tested by Netter and Levaditi is as follows (translation from the French):

"CASE 3.—Henriette G., sister of Emile (see below), was with a nurse in L'Aveyron, returning in good health Dec. 15, 1909. Three weeks after her return the child fell off, lost appetite, became cross, showed less disposition to run about, complained of pain behind the knees when the legs were touched, and of the arms when they were pulled. These symptoms, which attracted the attention of Dr. Erghischoff, caused no uneasiness to the rather heedless parents, who refused to show us the child on February 7. The mother thought the child was suffering from *mal du pays*. Whatever the trouble, it all disappeared in the course of three weeks. The child regained her normal appearance and showed every appearance of health the latter part of March."

A brother, Emile, 6 years old was taken ill either a few days before or a few days after this child (the dates given are not clear as to the sequence of cases) with a typical attack of acute anterior poliomyelitis, resulting in lasting paralysis of a leg and an arm.

The experiments which we here report are similar to the above experiment of Netter and Levaditi, undertaken on a more extensive scale, designed to throw some light on the diagnosis and epidemiologic significance of a class of cases encountered by one of us,[7] in a field study of acute anterior poliomyelitis at Mason City, Iowa, and other points in that state during the summer of 1910.

*From the Hygienic Laboratory.

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In Mason City, Iowa, a town of about 14,000 inhabitants, there occurred from April to September, 1910, forty cases of undoubted acute anterior poliomyelitis, resulting in typical flaccid atrophic paralysis. At the same time there occurred a considerable number of cases of illness with symptoms suggestive of the early symptoms of acute poliomyelitis, but not resulting in paralysis, and often of such mild character as not to come under the observation of any physician.

REPORTS OF CASES

Blood-specimens were obtained in the latter part of November from a number of these cases, whose clinical histories are given in abstract below. For the histories in several of these cases we are indebted to various physicians of Mason City. Other patients were not seen by any physician during their illness and the only histories available were such as could be given several weeks later by the patients and their families.

Case 1.—*Control.*—Reported by Dr. C. M. Swale, Mason City. C. T., male, aged 48, laborer, was taken sick June 24 with a chill, fever, pains in the legs and back, stiffness of neck, some delirium. On the third day there was paralysis of both thighs and legs and both upper arms, and partial paralysis of the forearms. When last seen, Nov. 24, 1910, the lower limbs and upper arms were still paralyzed, flaccid and atrophic, but showing some improvement. This is a typical case of acute anterior poliomyelitis, included in our series as a control.

Case 2.—Reported by Dr. B. F. Weston, Mason City. Mrs. W., 22, waitress, was taken sick the latter part of June, 1910, with fever and indefinite general symptoms. After several days she became paralyzed in both lower limbs. She was admitted to a hospital about one week after onset. She was said to have had, at that time, a flaccid motor paralysis of both lower extremities which, however, became spastic within a few days. When the patient was seen the latter part of July, 1910, both legs and thighs were quite spastic. No active motion was possible except of the toes and slight flexion of the left knee. Passive motion was limited to partial flexion of the thighs and slight flexion of left knee—almost none of the right knee. The patellar reflexes greatly exaggerated on both sides; ankle-clonus on right side; sensation for touch and pain was normal. Examination was otherwise negative. The patient's general health was good.

Nov. 25, 1910, the left leg could be moved, but rather awkwardly. The right leg showed little, if any, improvement. Patellar reflexes were still exaggerated, more so on the right side. There was no ankle-clonus and no atrophy.

The spasticity of the paralyzed limbs, exaggeration of reflexes and absence of atrophy in this case indicated a lesion in the upper motor segment, either in the motor cortex of the brain or in the pyramidal tracts of the cord. The case was included in our series in order to ascertain the diagnosis, since it represents a rare clinical type of poliomyelitis, the diagnosis of which has always been uncertain, and whose occurrence has been a matter of some dispute.

Case 3.—Reported by Dr. C. E. Dakin, Mason City. H. G., a woman aged 23, nurse, was taken sick suddenly June 24, after a long walk in the hot sun, with violent occipital headache, vertigo and stiffness of the neck, causing some retraction of the head. She had chilly sensations, vomited, was extremely prostrated, and felt "crazy." Temperature was normal, pulse 80. The next day she had pains in the legs and back, with tenderness along the spine, and was very restless. There was no further vomiting; the patient was constipated. The third day she complained of cramps and a sensation of numbness in the left leg, and found, on attempting to get out of bed, that this leg was weak. These symptoms gradually improved, so that in a week the patient was able to be up with no sign of paralysis. The spine, however, remained tender for several days and sensitive to jarring. A week later she had a slight recurrence of similar symptoms, with a pharyngitis.

There was no elevation of the temperature at any time in this case, which was seen daily by the attending physician. The patient knew of no direct association with cases of poliomyelitis, within a month prior to her illness. She was, however, at the time of her illness living at the house of a physician who had recently attended patients who were suffering from this disease.

Case 4.—Dr. C. E. Dakin, of Mason City, gives the following account of his own illness, the nature of which was hardly suspected at the time. He was taken sick about July 1, 1910, the onset being gradual. He lost appetite, was constipated, had a coated tongue and foul breath. He suffered from a persistent occipital headache, not relieved by treatment, and from severe pains in the upper dorsal and lumbar region. The neck was stiff, so that it was painful to bend the head forward although it could be rotated without pain. No retraction of the head was noted, but it was found that the most comfortable posture was lying on the back with the head extended over a pillow placed under the shoulders. He felt extremely nervous, irritable and inclined to worry over trifles. There was no elevation of temperature. These symptoms, which reached their height about July 7, gradually subsided within the next week, except the general nervousness and irritability, which persisted a good deal longer. During this time Dr. Dakin was not confined to bed, and continued his practice.

TABLE 1.—INOCULATIONS IN SERIES 1 *

Monkey No.	Serum from	Description of Case	Age	Result in Monkeys
16	Case 1 (control)	Frank poliomyelitis	Adult.	Has remained well
17	Case 2	Suspected poliomyelitis with spastic paralysis.	Adult	Has remained well.
18	Case 3	Suspected poliomyelitis without paralysis (abortive).	Adult.	Has remained well.
19	Case 4	Suspected poliomyelitis without paralysis (abortive).	Adult.	Has remained well.
20	Case 5	Suspected poliomyelitis without paralysis (abortive).	Adult.	Has remained well.
21	Case 6	Suspected poliomyelitis without paralysis (abortive).	Adult	Has remained well
25	Case 7 †	Suspected poliomyelitis without paralysis (abortive).	16	Paralysis all extremities on sixth day.
26	Case 8 †	Suspected poliomyelitis without paralysis (abortive).	Child.	Paralysis all extremities, trunk and neck.
22	Case 9	Suspected poliomyelitis without paralysis (abortive).	Adult.	Has remained well.
23	Case 10	Suspected poliomyelitis without paralysis (abortive).	Adult.	Has remained well.
24	Case 11 †	Suspected poliomyelitis without paralysis (abortive).		Paralysis all extremities on eighth day.
27	Control JR	Normal.	Adult	Paralysis of hind legs tenth day, all extremities twelfth day; chloroformed.
27A	Control JR	Normal.	Adult.	Paralysis of all extremities tenth day.

* Each monkey inoculated intracerebrally Nov. 30, 1910, with 0.5 c.c. of a mixture of 5 per cent. emulsion of fresh spinal cord of Monkey 15† 0.5 c.c.; serum to be tested, 0.5 c.c.; fresh normal serum, 0.1 c.c. Mixtures allowed to stand one hour at 37 C. and twenty hours at 15 C. before injection. † Refers to same serum in Series 2.

A child of his family had an attack of acute poliomyelitis with slight peroneal paralysis in May. Also about June 23 Dr. Dakin attended a child suffering from what was clearly an abortive attack of acute poliomyelitis.

CASE 5.—The patient, Mr. S., aged 30, merchant, in Mason City, was not seen by a physician. The history was obtained some two months later from the patient and his family. He was taken sick rather suddenly about June 19, with headache, pain in neck, back and legs. The temperature was not determined. The patient felt ill for a week or more, but was not confined to bed; thought he had the influenza. For more than a week after recovery one leg remained weak and "rheumatic." No previous association with any case of poliomyelitis was known. About three weeks later a 3-year-old son (the only child of the family) developed a typical case of poliomyelitis, resulting in paralysis of the left thigh and leg.

CASE 6.—Reported by Dr. C. E. Dakin, Mason City, who first saw the patient October 25. E. D., 45, farmer, residing about 7 miles northwest of Mason City, about Oct. 16, 1910, began to feel fleeting, neuritic pains in various parts of his body. There was no special pain, tenderness or stiffness of neck or back. A chronic indigestion became much aggravated, causing gastric distress, especially after eating. The patient lost appetite, and was constipated; the tongue was coated and breath foul. He was unusually nervous, irritable and sleepless. He improved gradually, returning to his usual state of health within two or three weeks.

A boy living in the same house was taken sick with acute poliomyelitis Oct. 10, 1910, and died October 16. The other two members of the household remained well at this time; but, as stated in connection with Case 7, both had suffered somewhat similar attacks about August 1.

CASE 7.—P. G., a girl aged 16, living about seven miles northwest of Mason City, was taken sick July 11 with headache, slight backache, pains in the knees, nausea and vomiting; diarrhea; fever doubtful. There was no stiffness of neck. The patient was in bed part of one day. Felt ill and unusually weak for several days thereafter.

There was no known contact with any case of poliomyelitis prior to onset. There had, however, been a considerable gathering of neighbors at this house one week prior, and the patient and other members of the family went back and forth to Mason City, where poliomyelitis prevailed at that time.

This was the first of a group of generally similar cases occurring in this and neighboring families. Other cases of similar illness occurred in this same family as follows: sister, aged 6, July 14; two sisters aged 7 and 8 respectively, July 15 or 16; brother, aged 10 (Case 8), July 22; father, aged 49 (Case 9), August 3. Two other members of the family, one adult and one aged 13, remained well.

It was stated that cases of similar illness had occurred about the same time in several neighboring families, all of which had been associated more or less closely with these cases, viz.:

Family B.—One case, adult, July 29.

Family W.—Five cases, about August 1.

Family D.—Two cases about the same time. This is the family of Patient 6. The two who were sick at this time remained well during the subsequent illnesses of the other members of the household.

Family M.—One case, date not known.

Family E. B.—One case, some time after August 15.

Family C. B.—Two cases (one of them Case 10), July 31

CASE 8.—R. G., brother of Patient 7, aged 10, was ill with sudden onset about July 22; had fever, sore throat, headache, pains in neck, back and limbs; was at first restless, then drowsy. After staying in bed one day he felt better and got up, but still had pain in the back. He fell down several times this day, apparently because of weakness of the legs. The third day he felt well, except for weakness, which gradually disappeared.

CASE 9.—W. G., father of Patients 7 and 8, aged 49, farmer, first noted, August 3, while driving his wagon, that the jolting caused him pain all over. Later in the same day he had a headache, chilly sensations and fever. The pain continued all day, chiefly in the back, neck and shoulders. The patient was constipated and had no appetite. The second day his ankles felt stiff and sore, and he felt extremely weak and uncertain on his legs. After the second day he felt much better, except for the weakness, which gradually improved, leaving him in his usual health

CASE 10.—Reported by Dr. C. E. Dakin, Mason City. C. B., aged 48, carpenter, about July 31 began to have an occipital headache, was constipated, had no appetite; felt very weak, tired and faint. He was troubled at times with vertigo, brought on by sudden turning of the head. The cervical and dorsal spine was tender. The headache and vertigo were worse when in the sunlight. There was some photophobia. The pupils were contracted and reacted sluggishly to light. Reflexes otherwise normal. These symptoms continued for about a week and for a much longer time the patient suffered from indigestion and continued to feel weak and easily tired.

The wife of this patient was taken sick about the same time with practically identical symptoms. These two had been between July 14 and 31, to a house where a child was sick at the time with a pretty definite abortive attack of poliomyelitis, one of them had also been to a house where two sisters of Patients 7 and 8 had visited while sick. A member of the household of Patients 7, 8 and 9 was said to be a frequent visitor at the house of Mr. B. He and his wife are the family "C. B." mentioned under Case 7.

CASE 11.—Reported by Dr. Fred Albert, Mason City. J. A., male, aged 7, residing on a farm about 5 miles northeast of Mason City, was taken sick suddenly June 30 or July 1, 1910. When seen by Dr. Albert, July 2, he had a temperature of 102.5

TABLE 2.—INOCULATIONS IN SERIES 2.*

Monkey No.	Serum from	Description of Case	Age	Result in Monkeys
30	Control JFA ‡	Normal	Adult.	Has remained well to date.
31	Control CHI	Normal	Adult.	Paralysis and death fifth day.
32	Control WHF	Normal	Adult.	Paralysis, forelegs and neck, thirteenth day.
33	Control W ‡	Normal	Child.	Very doubtful, slight illness; sixth to twelfth day loss of appetite, nervousness, excitability, slight irregular rise of temperature.
34	Control R ‡	Normal	Child.	Very doubtful, slight illness; sixth to twelfth day; symptoms as above but less marked.
35	Case 11 †	Suspected abortive case.	Child.	Has remained well to date.
36	Case 7 †	Suspected abortive case	16	Paralysis of all extremities ninth day; died ninth day.
37	Case 8 †	Suspected abortive case	Child.	Has remained well to date.

* Conditions of experiment the same as in Series 1, except that virus used was a 1 *per cent.* emulsion of fresh cord (of Monkey 29). Inoculations Dec. 24, 1910. † Refers to same serum in Series 1. ‡ Refers to same serum in Series 3.

TABLE 3.—INOCULATIONS IN SERIES 3.*

Monkey No.	Serum from	Description of Case	Age	Result in Monkeys
38	JFA (see monkey 30) ‡	Normal	Adult.	Paralysis of all extremities thirteenth day; chloroformed.
39	R (see monkey 34) ‡	Normal	Child.	Paralysis of all extremities tenth day; chloroformed.
40	W (see monkey 33) ‡	Normal	Child.	Paralysis of neck and forelegs tenth day; chloroformed

* Conditions of experiment the same as in Series 1, using a 5 *per cent.* emulsion of fresh spinal cord (of Monkey 9). Inoculations Jan. 12, 1911. This series is a control on Series 1. ‡ Refers to same serum in Series 2.

F., had a severe occipital headache, pain and tenderness of the spine, and some inflammation of the tonsils. The next day the temperature was normal and the headache and pain and tenderness of the spine had disappeared.

Two other children in the same house were taken sick between June 29 and July 1 with similar symptoms. One recovered within a few days, while the other developed typical poliomyelitic paralysis of the arms.

Cases 3 to 11 inclusive are representative of a class of cases which were reported to be quite common in and around Mason City during the epidemic of poliomyelitis. While there is a considerable variety in their symptomatology there is, in most cases, a fairly distinct syndrome, viz., headache, more commonly occipital; pain and tenderness along the spine; neuritic pains and hyperesthesia of the limbs; a marked degree of restlessness or irritability; prostration out of proportion to the severity of the other symptoms; some gastro-intestinal derangement (anorexia, nausea, constipation or diarrhea); occasionally other symptoms of nervous derangement, as vertigo, photophobia, delirium, muscular paresis, disordered reflexes. The temperature was definitely determined to be normal in Cases 3, 4 and 10, and found elevated (102.5° F.) in Case 11. In the rest of the cases no exact information is available, as the patients were not under the observation of a physician during the acute stage. Dr. Dakin,[8] of Mason City, who has reported elsewhere 36 cases of poliomyelitis, including Cases 3 and 10 of the above series, describes, as very characteristic, a staring, terror-stricken facial expression, which he noted also in Cases 3, 6 and 10 of our series.

Blood-specimens were obtained from all these cases by venepuncture during the latter part of November. The serum was withdrawn after coagulation and kept in closely stoppered sterile bottles. Repeated tests showed all specimens of serum to be sterile when used.

The virus used was originally obtained from the Rockefeller Institute for Medical Research through the courtesy of the director, Dr. Simon Flexner. It had been propagated through a long series of monkeys at the Rockefeller Institute and found very uniformly virulent. We have found it virulent for all the fresh monkeys which we have inoculated other than those mentioned in the following series.

SERIES 1.—Monkey 15, inoculated with the above virus, developed complete paralysis of the hind legs on the seventh day; it was chloroformed and necropsy performed the same day. A 5 per cent. emulsion of the fresh spinal cord was prepared in normal salt solution, and filtered through paper to remove gross particles. One-half cubic centimeter of this emulsion was added to an equal amount (0.5 c.c.) of each of the serums to be tested.

To each mixture was added 0.1 c.c. of fresh normal human serum (J. R.). As controls, two mixtures were made with normal human serum (J. R.). The mixtures of serum and virus were kept 1 hour at 37 C., then placed over night (about 20 hours) at 15 C. A series of monkeys was inoculated in the brain, each monkey receiving 0.5 c.c. of one of the mixtures of virus and serum, Nov. 30, 1910.

The two control monkeys receiving the mixtures of normal serum and virus developed typical paralysis on the eighth and tenth days respectively.

The monkeys receiving the mixtures of virus with the serum of Patients 7, 8 and 11 also developed equally typical paralysis on the seventh, fifth and seventh days respectively. All the others have remained well up to the present time (Feb. 4, 1911).

It will be noted that the three specimens of serum which failed to affect the virus were from young persons (Patient 7, aged 16; Patient 8, aged 10; Patient 11, aged 7). The remainder of the serums were from adults. This suggested two possibilities:

1. The serum of normal adults may have considerable germicidal properties for the virus.

2. The serum of children, even when they have had an attack of poliomyelitis, may not develop germicidal antibodies; or may develop them in smaller amounts than the serum of adults.

SERIES 2.—To test these hypotheses, we inoculated another series of monkeys with exactly the same technic, except that, instead of a *5 per cent.* emulsion of spinal cord we used a *1 per cent.* emulsion (fresh cord of Monkey 29, inoculated December 13, paralysis of hind legs December 23; chloroformed and cord removed December 23).

In this series we used again the serum of Patients 7, 8 and 11, and for controls took the serum of three normal adults (J. F. A., C. H. L., and W. H. F.) and of two normal children (R. and W.). Granting an equal concentration of the virus in the two cords used in series 1 and 2 (Monkey 15 and Monkey 29), each specimen of serum would now be mixed with the same *volume* of emulsion as in Series 1, but with *one-fifth of the amount of virus*. Also, the amount used in inoculation (0.5 c.c.) would represent only one-fifth of the amount of virus inoculated into the animals of series 1.

The results in this series were somewhat irregular. The monkeys receiving the serum of two of the normal adults (C. H. L. and W. H. F.) developed poliomyelitis on the fifth and thirteenth days respectively. The monkey which received the serum of Patient 7 developed paralysis of all extremities on the eighth day. None of the other monkeys, including those which received the serum of Patients 8 and 11, the serum of one normal adult (J. F. A.), and of the two normal children, have developed paralysis up to this time. Two of the monkeys, however (those receiving the serum of the two normal children), appeared about a week after inoculation to have an indefinite illness, manifested by slight rises of temperature, loss of appetite, nervousness, and undue excitability. Whether these may be considered abortive attacks of poliomyelitis is extremely questionable.

These results would indicate that in this series we had approached the limit of proportions in which normal human serum may exert a neutralizing effect on the virus of poliomyelitis, or else had approached the minimum effective amount of virus for inoculation. They indicate pretty clearly that the serum of Patient 7 had no unusual germicidal properties, but leave the other two cases (8 and 11) in doubtful status.

SERIES 3.—Three specimens of normal serum having been found capable of neutralizing the virus in the proportion used in Series 2, that is, against equal volumes *of a 1 per cent.* emulsion of virus, it remained to test these three specimens again under the conditions of Series 1. Accordingly we inoculated another series of monkeys, using a *5 per cent. emulsion* of virus (fresh cord of Monkey 9, inoculated Jan. 7, 1911; died fourth day; gross and histologic lesions typical).

The serums used were the same specimens which had shown neutralizing properties in Series 2, viz., from one normal adult (J. F. A.) and two normal children (R. and W.). The technic and proportions were exactly as in Series 1.

All three monkeys came down with typical paralysis between the eighth and the twelfth days.

The three series of experiments are presented in the accompanying tables.

SUMMARY

The results of these experiments may be summarized as follows:

Normal Human Serum.—The results in Series 2 indicate that normal human serum may have a germicidal action on the virus of poliomyelitis. If this is so, however, the action has quantitative limits which clearly differentiate it from the action exercised by the serum of persons who have had poliomyelitis as shown in

8. Dakin, C. E.: Iowa Med. Jour., Nov. 15, 1910, p. 229.

Series 1 and 3. No appreciable difference has been demonstrated between the normal serum of adults and of children in regard to their action on the virus.

Serum of Patients Who Have Recovered.—As shown by the workers above cited and by our results, the serum of persons who have recently recovered from frank attacks of poliomyelitis exhibits a germicidal action on the virus considerably greater than that exhibited by normal serum. Serum from a person suffering from paralysis of spastic type showed the same properties, thus confirming the clinical evidence that acute poliomyelitis may cause paralysis of this type.

The serum of six out of nine patients (66.7 per cent.) who had recently recovered from suspected poliomyelitis without paralysis (abortive cases) showed the same germicidal action as the serum from a frank case of poliomyelitis.

In the serum from the other three suspected abortive cases of poliomyelitis we were unable to demonstrate any germicidal property beyond that shown by normal serum. These three specimens of serum were all obtained from young persons. These three cases clinically resembled poliomyelitis more than did some of the adult cases; and the symptoms were, on the whole, equally severe. The following possibilities suggest themselves:

1. The cases may not have been poliomyelitis.

2. They may have been poliomyelitis, but, if so, antibodies were either formed in less amount, or disappeared more rapidly than in the adult cases.

The experimental evidence on which the specificity, constancy and quantitative relations of this reaction must be estimated is scant. So far as it goes, however, it justifies the inference that the reaction as demonstrated in our experiments is specific.

We feel justified in concluding that the diagnosis of acute poliomyelitis has been established in six of our nine suspected abortive cases. The diagnosis in the other three is not cleared up by the experiments. The clinical and epidemiologic evidence of poliomyelitis in these cases must be weighed against the absence of a serum-reaction of unknown constancy.

The facts here presented have a significant bearing on the diagnosis and epidemiology of acute anterior poliomyelitis.

DIAGNOSIS

The generally accepted criterion for diagnosis of poliomyelitis has been the development of paralysis or at least definite weakness. It has been urged that the attempt to make a diagnosis on any clinical evidence less than this would result in hopeless confusion; and this is partly true in the present state of our knowledge of the disease. In the cases of our series, however, poliomyelitis had been suspected on clinical evidence alone and confirmed by biologic test in 66.7 per cent., a fact which would indicate that the diagnosis of abortive cases of this class is not wholly a matter of guess-work, provided special attention has been directed to poliomyelitis, as was the case in Mason City. No laboratory diagnostic methods of demonstrated reliability and universal application have been evolved. The early examination of cerebrospinal fluid is the most reliable laboratory method at present known. Lumbar puncture for diagnosis is hardly justifiable, however, unless some consideration of the safety, either of the patient or of the community, makes an accurate diagnosis of special importance. Total and differential leukocyte counts offer a promising aid to diagnosis, but much more work will be necessary to establish the reliability of this method. But even in the absence of any specific diagnostic methods, much light can doubtless be thrown on the subject by a careful and conservative study of all cases which may reasonably be suspected of being cases of poliomyelitis, paying especial attention to examination of the nervous system and to association with cases of frank poliomyelitis.

Until the clinical recognition of poliomyelitis is placed on a more reliable basis there will remain an indeterminable factor in its epidemiology, a factor whose significance can only be surmised at present. Conclusions from epidemiologic data will have to be correspondingly conservative.

A more detailed discussion of abortive cases, as observed in Iowa, and of their epidemiologic relations will be reported in a forthcoming bulletin of the Hygienic Laboratory.

Therapeutics

PROPHYLAXIS OF SCARLET FEVER

Every physician, no matter how limited or specialized he may attempt to make his practice, is likely to encounter a case of scarlet fever, and to have his opinion asked in regard to what should be done to prevent the transmission of the disease to others. Even if he does not personally encounter the disease, he cannot avoid being asked the same question, apropos of some case he has not seen, by an anxious mother or a father seeking for positive information from one whom they believe to have the knowledge of an expert. It reflects no credit on his scientific attainments, it does not increase the confidence in him of his patients, if he cannot state promptly, clearly, concisely and positively what should be done by the family in which the disease occurs to prevent its spread to other individuals, what the family which does not have it, but is afraid of getting it, should themselves do, as well as what they have a right to expect others to do, in order to prevent the spread of the disease.

To the striking development of knowledge of sanitary science and preventive medicine which has taken place during the last three or four decades we are indebted for the wider recognition of the fact that scarlatina, scarlet rash and scarlet fever are synonymous terms, that they do not indicate different diseases, but one and the same disease. Formerly, many of the laity and not a few physicians believed, or at least said, that scarlatina and scarlet rash were different from scarlet fever. But numerous careful observations have shown, and the profession generally has learned, that while scarlet fever may be, and often is, a very serious disease with high temperature, severe sore throat, intense and widely spread eruption, followed by copious desquamation, the fever may be slight or entirely absent, the throat may not show more than slight congestion, the eruption, if not entirely absent, may be not very pronounced in appearance, not widely spread over the body and of rather transient duration, while the desquamation may be so slight as to be hardly recognizable.

Furthermore, it is now generally recognized not only that the very mild cases may be followed by the most serious sequelæ which are observed after the severe forms of the disease, and particularly by inflammation of the kidneys, but also that severe forms of scarlet

Noteworthy Papers on Viral Diseases

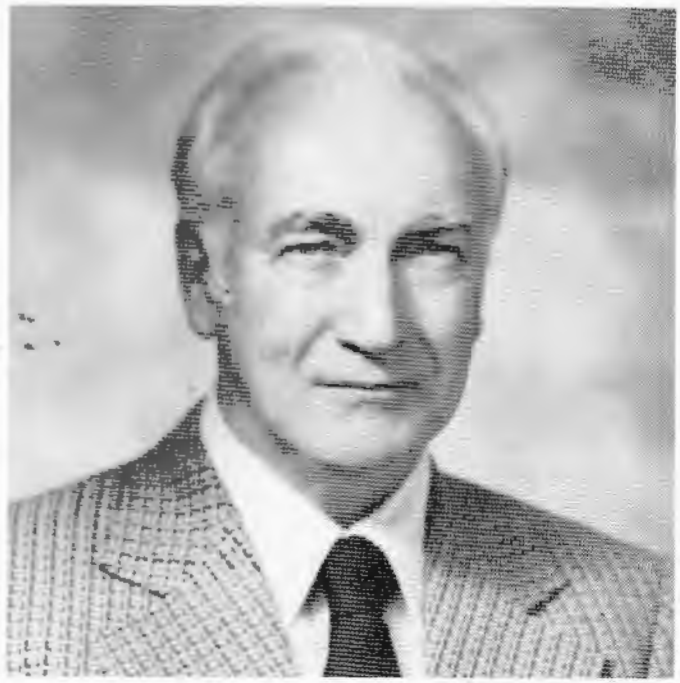
Dorland J. Davis

In the last decade of the 19th century when many bacteria were found to cause disease in many animals and plants, it was discovered that some biological materials were still infectious after being passed through a porcelain filter that removed all bacteria. These infectious agents came to be known as "filterable viruses" and included those of smallpox, vaccinia, yellow fever, foot and mouth disease of cattle, and other animal and plant infections. They were also invisible by the usual stains in a light microscope. Among these infections was poliomyelitis then commonly called infantile paralysis, which was beginning to occur in epidemic form in Europe and the United States. Physicians at the Hygienic Laboratory of the U.S. Public Health Service were called upon for assistance in understanding this disease and in the attempts to control it. Thus poliomyelitis was the first viral disease investigated by medical scientists at the Hygienic Laboratory.

Studies by Anderson and Frost demonstrated "specific immune bodies" in blood serum of non-paralytic cases in 1911, one of the earliest attempts to understand immunity in this infection. This paper is noteworthy because it demonstrates for the first time laboratory evidence of polio infection in persons with non-paralytic disease (abortive cases).[1] Written by two very influential medical scientists at the Hygienic Laboratory, it linked experimental work with clinical and epidemiological observations. A few years later Frost described in detail the clinical course and epidemiology of outbreaks occurring in Iowa and Cincinnati, and with Leake an outbreak in Buffalo and Batavia, NY.[2] Leake reported a winter epidemic in West Virginia.[3]

Although the infectious agent of poliomyelitis could be propagated in monkeys, investigation was hindered by the lack of a convenient and cheap experimental animal until Armstrong in 1939[4,5] adapted the Lansing strain to cotton rats and then to laboratory mice. This opened the way to quantitative measurement of virus and neutralizing antibody on a large scale. Progress was rapid thereafter as two other strains were also adapted to mice. Eventually the polio virus was cultivated in tissue culture, leading to the development of effective vaccines.

Encephalitis outbreaks appeared in the early 1930s and National Institute of Health scientists were called to investigate. During the course of the 1933 epidemic in St. Louis, Armstrong and Lillie[6] recovered a virus that produced a lymphocytic choriomeningitis in monkeys but was shown not be the cause of the epidemic. This virus, termed LCM (lymphocytic choriomeningitis), was found



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to cause disease primarily in rodents (house mice) and in humans exposed to infected mice (*La Maladie de Armstrong*). Later Bengtston and Wooley[7] cultivated the virus in embryonated eggs. This virus is now classified with the arenavirus family. Members of this family are responsible for some of the most severe infections in man, such as Bolivian hemorrhagic fever.

During World War II, most NIH investigations were oriented to war-time problems. By then better techniques for laboratory work had been developed such as the use of embryonated chicken eggs and improved serological methods. Mumps was always an important disease in military recruits. Habel[8] succeeded in cultivating the virus in embryonated eggs and devised serological tests for its presence. Later vaccines were developed and are now widely and effectively used.

Measles (rubeola) was also an important war-time disease. In 1911 Anderson and Goldberger[9] at the Hygienic Laboratory had transmitted the infection with great difficulty to monkeys by contact. Progress and a vaccine, however, had to await the development of specific tissue culture techniques in the 1950s. Rubella was investigated by Habel[10] who succeeded in passaging

an isolate through chick embryos and then to monkeys. In the 1960s, an effective vaccine was prepared by several laboratories that reduced the threat of infant malformation due to this infection.

Following World War II, interest turned to respiratory diseases such as influenza and the common cold. A group of agents termed the Coxsackie viruses was intensively investigated. Heubner and his associates[11] in 1951 showed that herpangina, an illness characterized by fever, sore throat, and anorexia that mainly affected children, was caused by several types of Group A Coxsackie virus, which could be isolated in suckling mice. They also implicated Coxsackie B with cases of pleurodynia in Texas.[12] Later Coxsackie A-21 was found to be an important cause of acute respiratory disease in military populations which led to development of a vaccine that reduced the burden of this infection in training camps.[13]

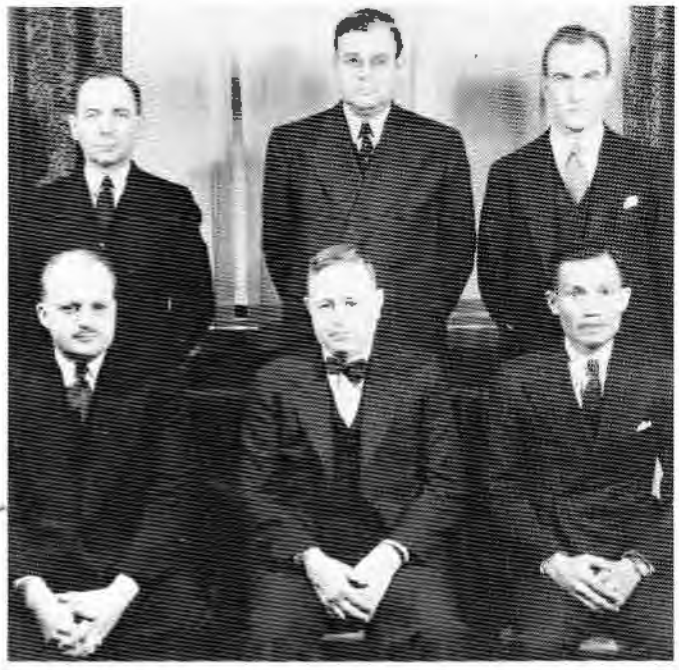
NIAID scientists continued to investigate respiratory infections, and Rowe and others were the first to isolate an adenovirus from patients.[14] Virus was found in adenoid tissue that was observed to degenerate in successive tissue culture passages. A large number of types have now been recovered from tonsils and adenoids. Several types, 4, 7, and 14 are frequently associated with respiratory diseases[15] and type 8 with keratoconjunctivitis. Rowe, Heubner, Bell, and their associates[16] clarified the role of the adenoviruses in respiratory disease and described the epidemiology and clinical characteristics of these infections.

Further studies of these viruses by Heubner[17,18] and others demonstrated virus-free adenovirus antigens in animal tumors that led to extensive investigations on oncogenicity. Rowe[19] secured evidence for a genetic hybrid between adenoviruses 7 and SV 40, a papovavirus of monkeys. This work was expanded to recombinants of adenovirus hybrids. Heubner and associates[20] found oncogenic effects in hamsters when injected with human adenovirus types 12 and 18.

The influenza epidemics of 1957 and 1968 stimulated intense investigation of the antigenic characteristics of influenza type A virus with the goal of improved vaccines. Murphy and associates[21] developed temperature sensitive mutant influenza strains that may eventually lead to an effective and safe live virus vaccine.

Chanock and his group also investigated the cause of infantile croup and other respiratory childhood infections. They first established the etiologic role of cytopathic myxoviruses, parainfluenza type 1 and type 3, in these conditions.[22,23] NIAID scientists[24] also studied clinical conditions caused by the rhinoviruses, the cause of the common cold and with others showed that the group was composed of over 100 different serological types.

Hepatitis had been differentiated into infectious (A) and serum (B) soon after the extensive experience of World War II. Hepatitis B, the most lethal, commanded principal attention particularly after the identification of the Australia antigen as a component of the infectious agent. Gerin, Holland, and Purcell[25] contributed importantly to its purification from human serum and to biochemical studies of its proteins.



Dr. Davis, standing on right, in 1937 at Johns Hopkins School of Public Health.

Hepatitis A, a frequent cause of epidemics, particularly in tropical areas, is widely prevalent but less lethal than B. Feinstone, Kapikian, and Purcell,[26] using immune electron microscopy were able to visualize HAV viral particles clumped together with immune serum in the stools of hepatitis patients and to measure HAV antibodies. This provided an excellent means of diagnosis and a basis for epidemiological and natural history studies of human infection.

Usually following transfusions, some cases of clinical hepatitis occurred that could not be diagnosed as either A or B. These infections were called hepatitis non-A non-B and were found by Feinstone and associates to be transmissible.[27] They still form a major part of post-transfusion hepatitis as well as sporadic hepatitis.

In the early 1960s investigation of arbovirus infections began, based at a laboratory in the Panama Canal Zone known as the Middle American Research Unit or MARU sponsored by NIAID and the Walter Reed Army Institute of Research. One important result was the study of Bolivian hemorrhagic fever, a devastating disease in some rural areas of Bolivia and other South American countries. Johnson and his co-workers[28,29] recovered a virus from patients and from wild rodents living in human habitations. They called it Machupo and later studied similar agents from Africa. The group, including the LCM virus, is now known as the arenaviruses.

Continued study of respiratory viruses in children yielded infectious agents related to animal viruses. McIntosh[30,31] and his associates studied the antigenic relationships and pathogenicity of these coronaviruses, as they came to be called, and found they were a significant cause of childhood respiratory illness.

The most important cause of lower respiratory tract disease in infants and children is the respiratory synctial virus (RSV), first recovered in 1957 by Chanock and associates.[32,33] RSV is now considered to cause thousands of infant deaths in this country alone. Attempts to develop a vaccine through intensive research thus far have not been successful.

Kapikian extended the technique of immune electron microscopy to other agents and detected and identified an agent, the Norwalk virus, associated with acute non-bacterial gastroenteritis. These observations have led to great advances in knowledge of the common but heretofore poorly understood intestinal infections, especially in children. The Norwalk virus is now known to be responsible for one-third of all epidemics of non-bacterial gastroenteritis.[34]

At the Rocky Mountain Laboratory in Hamilton, Montana, where excellent animal facilities were available, Hadlow[35] studied the "slow virus" of scrapie, a sheep disease. He compared the condition to Kuru, a fatal disease in New Guinea natives that has been carefully studied by Gajdusek in recent years. Eklund, Kennedy, and Hadlow[36] later described the pathogenesis and viral replication of scrapie virus in the mouse. The "slow viruses" are now recognized as a distinct form of microbial pathogens that may be the cause of some chronic diseases.

The role of viruses in production of tumors has been of constant interest, beginning with studies of the polyoma virus of mice by Eddy and Stewart.[37] Habel[38] discovered a virus specific transplantation antigen on cells transformed by polyoma virus. Rowe, and associates[39,40,41] studied extensively animal leukemia and sarcoma viruses in tumors and the immunological reactions.

Underlying all the tissue culture work with viruses were the very careful studies by Eagle[42] of the specific amino acid requirements of a mammalian cell in tissue culture. It made possible the rapid development of lines of various cell types for cultivation of a wide variety of viruses. Eagle's defined media for tissue culture cells has been a major contribution to the study of viral diseases.

Detailed studies on the molecular structure of viruses and the processes of replication have laid a foundation for understanding and manipulating them. Moss and Salzman[43] analyzed the protein synthesis of vaccinia virus and the process of virus assembly. Sebring[44] and associates provided one of the first demonstrations of the structure of replicating DNA molecules. Shatkin[45] and his associates gave the first demonstration of a transcriptase activity in an RNA virus. Later Weir and Moss[46] began further investigation of molecular structure of vaccinia virus leading to important practical results in construction of a variety of immunizing agents on a molecular basis.

An ever expanding array of techniques, instrumentation and concepts has lead to the remarkable unfolding of our knowledge of viral diseases in the last 80 to 90 years and to effective methods of control of many of them. As demonstrated by the work cited here, scientists of the early Hygienic Laboratory and those in the current NIAID intramural program have played key roles in the world-wide explosion of medical science.

Dorland J. Davis, M.D., D.P.H.

Born in Chicago, Illinois in 1911, Dr. Davis received a B.S. degree from the University of Illinois in 1933, and an M.D. from Johns Hopkins University in 1937. Two years later, shortly before receiving a D.P.H. from Johns Hopkins University, he joined the Public Health Service as a researcher with NIH's Division of Infectious Diseases. His entire career was spent at NIH/NMI/NIAID, except for duty in North Africa for the Department of State from 1943 to 1944 as a member of a medical team investigating endemic diseases particularly malaria and typhus. In 1954 he was appointed Chief of the Laboratory of Infectious Diseases. His research interests in the 1940s were poliomyelitis, American trypanosomiasis (Chagas' Disease), viral hepatitis, aud psittacosis. In the late 1940s and early 1950s, he studied acute epidemic conjunctivitis in children, elucidating its cause, epidemiology and treatment. Dr. Davis next turned to studies on influenza. In the late 1950s he travelled to Central and South America, Alaska, and McMurdo Sound areas of Antarctica evaluating research opportunities and disease problems. In 1956 he was appointed NIAID's first Associate Director in Charge of Research, a position later called Director of Intramural Research. From 1964 until 1975 he served as Director of NIAID, retiring in 1975 after a 36 year career.

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TREASURY DEPARTMENT.
Public Health and Marine-Hospital Service of the United States.

HYGIENIC LABORATORY.—BULLETIN No. 43.
MARCH, 1908.

THE STANDARDIZATION OF TETANUS ANTITOXIN

(AN AMERICAN UNIT ESTABLISHED UNDER AUTHORITY OF THE ACT OF JULY 1, 1902)

BY

M. J. ROSENAU
and
JOHN F. ANDERSON.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1908.

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THE STANDARDIZATION OF TETANUS ANTITOXIN[a]

[*An American unit established under authority of the act of July 1, 1902.*]

By MILTON J. ROSENAU,
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and
JOHN F. ANDERSON,
Passed Assistant Surgeon, Assistant Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

INTRODUCTION.

There are now four methods of measuring the strength of tetanus antitoxin: (1) The German method described by Behring, (2) the French method of Roux, (3) the Italian method after Tizzoni, and (4) the American method described in this bulletin.

The European standards are admitted to be unsatisfactory and, for the most part, not accurate; further, they are complicated and difficult to carry out. The American method adopted officially under the law of July 1, 1902, for this country is the result of several years, work upon this subject in the Hygienic Laboratory and it is believed will commend itself for its simplicity, directness, and accuracy.

The standard toxines and antitoxins are preserved under special precautions to prevent deterioration and are tested against each other reciprocally, so that the least alteration in either may be detected.

While the unit is based upon the neutralizing value of an arbitrary quantity of antitoxic serum, the *antitoxin* is not issued to other laboratories for the purposes of test, as is the case with diphtheria. A stable precipitated tetanus *toxine*, the test dose of which has been carefully determined, is given out. All the tetanus antitoxic serums for use in man upon the American market are now measured against this same standard toxine and have therefore precisely comparable strengths.

The need of uniformity in standardizing this valuable prophylactic serum is evident from the table on page 11, from which it will he seen

[a] Manuscript submitted for publication March 4, 1908.

that before the establishment of an American standard the tetanus serums upon the market varied extravagantly in the unit strength claimed, and were, for the most part, comparatively weak in true antitoxic potency. Since the promulgation of this standard unit the serums on the market have decidedly greater antitoxic value. The feebleness of the foreign serums compared with those of American manufacture is also evident from the table.

The object of this bulletin is to describe in detail the methods used in preparing and using this standard. A discussion of the theoretical considerations is introduced for a better understanding of the principles involved.

AMERICAN UNIT, AND METHODS OF STANDARDIZATION.

DEFINITIONS.—"The immunity unit for measuring the strength of tetanus antitoxin shall be ten times the least quantity of antitetanic serum necessary to save the life of a 350-gram guinea pig for ninety-six hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health and Marine-Hospital Service."

The unit was thus officially defined October 25, 1907, in Treasnry Department Circular No. 61. This circular amended the regulations promulgated in accordance with the act approved July 1, 1902, entitled "An act to regulate the sale of viruses, serums, toxins, and analogous products in the District of Columbia, to regulate interstate traffic in said articles, and for other purposes."

Based largely upon the work on the standardization of tetanus antitoxin done in the Hygienic Laboratory, a special committee of the Society of American Bacteriologists, which met in New York December 27 and 28, 1906, made the following report, which was unanimously adopted:

> That tetanus antitoxin be standardized by the tetanus toxin furnished by the Public Health and Marine-Hospital Service. The unit is ten times the least amonnt of serum necessary to save the life of a 350-gram guinea pig for ninety-six hours against the official test dose of the standard toxin. The test dose is 100 minimal lethal doses of a precipitated toxin preserved nnder special conditions at the Hygienic Laboratory of the Public Health and Marine-Hospital Service. It was decided that the minimal immunizing dose for a case of possible infection through a wound should be 1,500 of such units. It was decided that after April 1 the new unit should be adopted by all producers of tetanus antitoxin.
>
> J. J. KINYOUN, *Chairman.*
> THEOBALD SMITH.
> HERBERT D. PEASE.
> E. M. HOUGHTON.
> JOSEPH MCFARLAND.
> M. J. ROSENAU.
> WILLIAM H. PARK, *Secretary.*

The American unit for measuring the strength of tetanus antitoxin may be defined as the neutralizing power possessed by an arbitrary quantity of antitetanic serum preserved under special conditions to prevent deterioration in the Hygienic Laboratory of the Public Health and Marine-Hospital Service. This arbitrary quantity now contains ten times the amount of tetanus antitoxin necessary to neutralize somewhat less than 100 minimal lethal doses of a standard toxine for a 350-gram guinea pig—that is, one-tenth of a unit mixed with 100 minimal lethal doses of the standard toxine contains just enough free poison in the mixture to kill the guinea pig in four days after subcutaneous injection.

The standardization of tetanus antitoxin does not differ radically from the standardization of diphtheria antitoxin. The toxines and antitoxins are measured against each other reciprocally, so that change or deterioration of either the standard toxine or the standard antitoxin may readily be determined. Duplicate toxines and antitoxins made from time to time will act as checks against deterioration of either the standard toxines or antitoxins.

The value of an unknown serum is measured indirectly through the toxine, using the L+ dose as the test dose. The L+ dose is the smallest quantity of tetanus toxine that will neutralize one-tenth of an immunity unit, plus a quantity of toxine sufficient to kill the animal in just four days.

The *toxine* and not the *antitoxin* is given out to licensed manufacturers and others interested for the purposes of standardizing their serums. The L+ or test dose of the particular toxine (A) now dispensed contains just 100 minimal lethal doses for a 350-gram guinea pig. This particular toxine is very stable and has not changed appreciably in two years. As soon as it alters or is exhausted the next toxine that will be issued may contain considerably more or less than 100 minimal lethal doses, but the test dose will contain precisely the same neutralizing power.

The antitoxic serum, for the purposes of this standard, was obtained from a single horse. The serum was reduced to dryness and ground to an impalpable powder. The powder so obtained is preserved in many vacuum tubes under the influence of pentaphosphoric acid. These tubes are kept in absolute darkness at a constant temperature of 5° C. Every two months, or oftener, one of these tubes is opened, dissolved in a solution of glycerin 66 parts and isotonic salt solution 34 parts, and tested.

While the tetanus antitoxin preserved in dry powdered form under the conditions above named is stable, duplicates of the antitoxins made from time to time so as to guard against loss or change will insure the permanence of the standard which has now been established.

METHODS.

In order to obtain reliable and comparable results, it is necessary to take into account all the factors concerned—the composition of the poisons, their concentration, the diluting fluid, length of time the mixtures are allowed to stand, the site of inoculation, etc. All the known factors have been considered in our method of standardizing tetanus antitoxin, so that it is our belief that we now have as accurate and satisfactory a standard for this antitoxin as is the case with diphtheria.

Careful regard must be had for the following points in order to obtain uniform results:

The diluting fluid.—Salt solution-containing 0.85 per cent chemically pure sodium chlorid sterilized by boiling is used for diluting both the toxine and the antitoxin.

Time and temperature.—The mixtures of toxine and antitoxin are kept one hour at room temperature in diffused light before injection into the guinea pig.

Amount.—A total of 4 c. c. of the toxine-antitoxin mixture is injected into the guinea pig. If the toxine-antitoxin mixture does not equal 4 c. c., salt solution equal in amount to the difference is used to wash out the syringe, so that the total amount injected and the pressure effects are always equal.

Concentration of the toxine.—One-tenth of a gram of the dried tetanus toxine (A) is dissolved in 166.66 c. c. salt solution. One c. c. of this dilution equals the test dose (L + dose).

Concentration of the antitoxin.—The dilution of the antitoxic serum should follow the tables on page 50.

Guinea pigs.—The guinea pigs should weigh 350 grams. Animals weighing from 300 to 400 grams are allowable in preliminary rough testing.

Site of injection.—The injection is always given subcutaneously into the tissues of the abdomen about the level of the umbilicus.[a]

Time of death.—The number of immunity units contained in the serum is determined from the amount given the animals that are living ninety-six hours after the inoculation of the mixtures.

An example of a test.—Carefully tare a weighing bottle, then add approximately 20 to 50 mg. of the dried poison. Again carefully weigh. Dissolve the toxine in the weighing bottle with salt solution (0.85) in the proportion of 0.1 gram of the dried poison to 166.66 c. c. of the salt solution. This proportion is used for the reason that each cubic centimeter of this solution will represent 0.000,6 gm. of the original dried poison (=100 MLD's). This proportion

[a] It is very important to inject the animals in the same place on account of the relation to the motor nerve endings and the distance to travel to the central nervous system.

is taken because it is very convenient in measuring out the test dose, which represents 1 c. c. of the solution. Thus:

44.5692 gm., bottle + toxine.
44.5300 gm., bottle.

.0392 gm., toxine.
0.1 gm. : 166.66 c. c. :: 0.0392 : x.
x = 65.33 c. c.

In other words, if the quantity of toxine placed in the weighing bottle should weigh, as in this instance, just 0.0392 gm., carefully deliver from an accurately graduated burette just 65.33 c. c. salt solution into the weighing bottle; and, as before stated, each cubic centimeter of this solution will be the L+ or test dose.

Now dissolve the serum of unknown value in accordance with the table of dilutions (page 50) and mix aliquot parts of the serum with the test dose of toxine, as follows:

TABLE No. 1.

No. of guinea pig.	Weight of guinea pig (grams).	Subcutaneous injection of a mixture of—		Time of death.
		Toxine (test dose).	Antitoxin.	
		Gram	c. c.	
1.....	360	0.0006	0.001	2 days, 4 hours.
2.....	350	.0006	.0015	4 days, 1 hour.
3.....	350	.0006	.002	Symptoms.
4.....	360	.0006	.0025	Slight symptoms.
5.....	350	.0006	.003	No symptoms

According to this series the guinea pig which received 0.0015 c. c. of the serum died in four days and one hour. Therefore 0.0015 c. c. of the serum contains one-tenth of an immunity unit, as the unit has been defined as ten times the least amount of antitetanic serum necessary to save the life of a 350-gram guinea pig 96 hours against the official test dose. This serum would, therefore, contain just 66 units per cubic centimeter.

On account of the importance of the L+ or test dose the following table is given showing the results of these tests with Toxine A.

TABLE No. 2.—*Showing the results of the test (L+) dose of tetanus toxine A from January 17, 1907, to April 7, 1908.*

Guinea pig No.	Weight in grams.	Date.	Amount of toxine A.	Amount of antitoxin.	Time of death.	
			Gram.		*Days.*	*Hours.*
306.....	290	Jan. 17, 1907	0.0006	1 unit.....	4	0
326.....	300	Jan. 24, 1907	.0006	1 unit.....	3	3
334.....	315	do.....	.0006	1 unit.....	3	7
387.....	350	Feb. 20, 1907	.0006	1 unit.....	4	19
388.....	350	do.....	.0006	1 unit.....	4	8
389.....	350	do.....	.0006	1 unit.....	4	16

TABLE NO. 2.—*Showing the results of the test (L+) dose of tetanus toxine A from January 17, 1907, to April 7, 1908*—Continued.

Guinea pig No.	Weight in grams.	Date.	Amount of toxine A.	Amount of antitoxin.	Time of death.	
			Gram.		*Days.*	*Hours.*
400	330	Feb. 25, 1907	0.0006	1 unit	4	21
401	315	do	.0006	1 unit	5	7
407	335	do	.0006	1 unit	4	16
408	315	do	.0006	1 unit	4	9
415	300	Mar. 5, 1907	.0006	1 unit	3	0
416	325	do	.0006	1 unit	3	10
422	300	do	.0006	1 unit	3	9
423	325	do	.0006	1 unit	3	10
430	290	Mar. 22, 1907	.0006	1 unit	4	11
431	290	do	.0006	1 unit	4	14
454	300	Mar. 28, 1907	.0006	1 unit	4	1
455	300	do	.0006	1 unit	3	18
509	300	Apr. 13, 1907	.0006	1 unit	3	17
510	295	do	.0006	1 unit	3	17
524	280	May 3, 1907	.0006	1 unit	3	18
525	290	do	.0006	1 unit	3	11
538	300	May 17, 1907	.0006	1 unit	3	20
539	320	do	.0006	1 unit	3	7
556	330	May 24, 1907	.0006	1 unit	2	20
557	350	do	.0006	1 unit	3	10
615	375	July 28, 1907	.0006	1 unit	2	22
616	380	do	.0006	1 unit	3	10
625	350	July 31, 1907	.0006	1 unit	3	13
626	350	do	.0006	1 unit	4	12
639	350	Sept. 6, 1907	.0006	1 unit	3	4
640	350	do	.0006	1 unit	3	2
654	325	Sept. 24, 1907	.0006	1 unit	3	17
655	370	do	.0006	1 unit	6	19
679	325	Nov. 11, 1907	.0006	1 unit	3	19
680	350	do	.0006	1 unit	4	0
682	350	Dec. 7, 1907	.0006	1 unit	3	15
684	360	do	.0006	1 unit	3	13
702	370	Dec. 19, 1907	.0006	1 unit	3	23
757	350	Apr. 7, 1908	.0006	1 unit	4	1
758	350	do	.0006	1 unit	3	18
759	350	do	.0006	1 unit	4	0

NECESSITY FOR THIS STANDARD.

We now know that when the symptoms of tetanus have developed the toxine has combined with the motor nerve cells and that the union between the cell and the poison is too strong for the antitoxin to break up. However, if the antitoxin is given in time the toxine is neutralized and rendered harmless. Tetanus antitoxin is therefore an exceedingly valuable prophylactic and for this purpose is coming into more general use both in human and veterinary practice.

Whether used as a prophylactic or curative agent it is important to use a potent serum. The law of July 1, 1902,[a] not only insures

serums of useful potency, but also establishes uniformity among the producers of tetanus antitoxin in America. The necessity of establishing uniformity for this product in American markets is evident from tbe following table:

TABLE No. 3.—*Showing the differences in the methods of testing tetanus antitoxin before the adoption of the American unit.*

Name.	Labeled.	Contents (cubic centimeters).	Units claimed according to special methods of standardization, per cubic centimeter.	Units according to the American standard, per cubic centimeter.
New York department of health, Albany (submitted by Dr. H. D. Pease for comparison).	One unmunizing dose (No. 2929F).		0.5	434
New York City department of health (submitted by Dr. W. H. Park for comparison).			700	166
H. K. Mulford Co. (bought on open market).	1,000,000 immunizing units (No. 2121).	10	100,000	77
Farbenfabrik vorm. Meister Lucius & Bruning, Hoechst, a/M (submitted for comparative tests).	Tetanus Antitoxin. 5 fach. Normal. Prüfungs dose = 1/500.			333
H. K. Mulford Co., No. 997) (submitted for tests).	60,000,000 units.....	10	6,000,000	90
Parke, Davis & Co.....			60.000	700
New York department of health, Albany (submitted by Dr. H. D. Pease for comparison).			.75	709
Serum Antitetanique, Pasteur Institute, 10 c. c. fluid.	Unit value not stated.	10	Not stated.	40
Serum Antitetanique, Pasteur Institute..	Unit value not stated.	10	Not stated.	40
Tizzoni, Antitossina del Tetano, No. 2912. Dry.	Unit value not stated.		Not stated.	[b] 83.3
Pasteur Institute, Serum antitetanique, II-7.	Unit value not stated.	10	Not stated.	66
Institute Bacteriologique de Lyon et du Sud-Est.	Unit value not stated.	10	Not stated.	(c)

[a] Dissolved in 26. c. c.
[b] This equals 833 units per gram of the dry serum.
[c] Less than 50.

Tetanus antitoxin is made by the following laboratories in the United States:

Parke, Davis & Co., Detroit, Mich. (license No. 1); H. K. Mulford Company, Philadelpbia, Pa. (license No. 2); Lederle Antitoxin

[a] An act regulating the sale of viruses. serums. toxins, and analogous products, etc. Approved July 1, 1902.

Laboratories, New York, N. Y. (license No. 17); New York State department of health, Albany, N. Y.; and the department of health of the city of New York, N. Y.

HISTORICAL REVIEW.

Compared with the major plagues of man lockjaw has always been a rare disease. It is on account of the peculiar and characteristic spasms that it early attracted attention. In the writings of Hippocrates cases of tetanus are described, diagnosed, and prognosis given. From the learned Aretaeus of Kappadoza we have a description of tetanus that holds until modern times.[a] Aretaeus described an opisthotonos, an emphrothotonos, and a tetanus, depending upon whether the muscles of the back, the abdomen or the general muscular system were affected. To this classification pleurothotonos was later added when the muscles of one side were especially affected.

Throughout the Middle Ages our knowledge of tetanus remained at a standstill. The symptoms of the disease so plainly indicated that the lesions are localized in the nervous system that, with the introduction of studies in pathologic anatomy, the brain, spinal cord, and nerves were studied to determine the seat and nature of the illness. It was not, however, until about the end of "the sixties" in the last century that experimental studies in wound infections began to throw light upon the chaos of theories. A review of the historical development of these theories is exceedingly interesting, for they mirror the prevailing thought upon the nature of disease, as it developed from that of evil spirits, through the "humoral" theory, the realm of miasm and noxious effluvia, to the germ theory.

It was long known that tetanus was a complication of wounds. Micheles, 1797, thought it was due to irritation of the peripheral nerves from foul secretions in certain wounds ("wound insults"). Tetanus could not escape the rheumatism theory, which has been such an alluring catch-all for symptoms and diseases difficult of explanation. In cases of wound tetanus in which no plausible explanation seemed possible it was called rheumatic wound tetanus. "Taking cold" was assigned its usual rôle here as elsewhere. When no assignable cause seemed at hand the disease was given the learned title "idiopathic tetanus."

About 1860 Heiberg and Rose, and Billroth and Spencer Wells[b] attached tetanus to the list of zymotic diseases and believed it due to a miasm, the spasms being caused by a poison in the blood similar to strychnine.

[a] A part of the historical data are taken from Von Lingelsheim's article on Tetanus in Kolle & Wassermann's Handbuch der pathogenen Mikroorganismen, vol. 2, p. 566–600.

[b] Billroth and Spencer Wells. Wiener med. Presse, 1869, p. 36.

E. Rose[a] 1870, described a particular form of tetanus following wounds in the area of the twelfth cerebral nerve which he called "head tetanus" or "tetanus hydrophobicus."

An important milestone in the history of tetanus was passed when Strümpell,[b] 1876, declared tetanus to be an infection. The first experiments to prove this view failed.

Arloing and Tripier[c] did not succeed in transferring the disease to dogs and rabbits by carrying over the blood and the pus from tetanus wounds.

Billroth[d] and Schultz[e] also obtained negative results with dogs.

Carle and Rattone,[f] 1884, were the first to obtain positive results in demonstrating the transmissibility of the disease. They injected a number of rabbits with the washings from the neighborhood of an acne pustule from which the tetanus originated. The rabbits were injected either into the sciatic nerve or into the muscles of the back. After an incubation period of two to three days 11 of the 12 rabbits showed typical tetanic spasms. They also succeeded in carrying the infection from animal to animal.

By injecting earth taken from different places into 140 mice, rabbits, guinea pigs, etc., in studying the microorganisms of soil (ground tetanus), Nicolaier,[g] 1884, produced symptoms of tetanus in 69 of them and always found a slender rod in the pus. Therefore he concluded that there exists a bacillus that causes tetanus in deep wounds of mice, rabbits, and guinea pigs. In cultures Nicolaier was unable to separate this bacillus from the other microorganisms with which it was associated, but inoculation experiments with mixed cultures produced tetanus.

The next year (1885) Nicolaier,[h] working under Flügge in the Göttingen Hygienic Institute, made noteworthy advances upon the subject. He showed that each injection into mice, guinea pigs, and rabbits caused a disease with tetanic spasms, while dogs were refractory. Nicolaier saw in the pus of the wounds this slender bacillus,

[a] Nicolaier, Arthur: Zur Aetiologie des Kopftetanus (Rose). Virch. Arch., vol. 128, 1892, p. 1–19.

[b] Strümpell: Ueber die Ursachen der Erkrankungen des Nervensystems. Arch. f. klin. Med., vol. 35, p. 14–15.

[c] Arloing and Tripier: Gaz. med. de Paris, 1870, p. 337.

[d] Billroth: Allgemeine chirurgische Pathologie und Therapie. Berlin, 1882, p. 504.

[e] Schultz: Ueber einer Kumulation von Tetanusfällen im Stadtkrankenhause zu Rostock. Rostock, 1876, p. 13.

[f] Carle and Rattone: Studio sull' enziologia del tetano (communicazione preventiva). Giorn. dell' R. Accad. di med. di Torino, Marzo, 1884.

[g] Nicolaier, Arthur: Ueber infectiosen Tetanus. Deut. med. Woch., vol. 10, 1884, p. 842–844.

[h] Nicolaier, Arthur: Beiträge zur Aetiologie des Wundstarrkrampfes. Inaug. dis-

which he considered the cause of the infection, but again was unable to obtain it in pure culture, although he carried on a mixed culture upon coagulated calf's blood serum.

Soon afterwards Rosenbach,[a] 1886, found a similar bacillus with a round terminal spore in a case complicating frost gangrene in man and transferred the infection to animals in the pus.

In 1889, with the aid of anaerobic technic, Kitasato[b] for the first time grew the bacillus in pure culture and by successful inoculation experiments finally proved that this bacillus was the real cause of tetanus. Kitasato succeeded in isolating the tetanus bacillus, first described by Nicolaier and later by Rosenbach, not alone by understanding anaerobic methods but by taking advantage of its resisting spore. This bacillus appears in the pus of wounds of man and animals having tetanus. It often forms spores in the pus, but if the pus is examined early it may contain spore-free rods. He planted the material containing the bacillus upon agar slants, which were incubated one to two days. The mixed cultures so obtained were heated one hour at 80° C. and then transplanted to agar plates. Kitasato designed for this purpose a special shaped apparatus in which hydrogen was used to replace the air.

Kitasato[c] further showed that the tetanus bacillus is not found in the heart's blood of mice dead of tetanus and therefore concluded that we are dealing with an intoxication and not with an infection.

It was in the following year, 1890, that Behring and Kitasato[d] published their epoch-making work upon the tetanus toxin and tetanus antitoxin, laying the foundation of serum therapy. On account of the great historical and practical importance of this document a transcription of its principal features is given:

The immunity of rabbits and mice which have been immunized against tetanus depends upon the power of the cell-free serum to render the toxic substances which are produced by the tetanus bacillus harmless.

Experiments show:

1. The blood of tetanus immune rabbits possesses the property of destroying the tetanus poison.

2. This property is destroyed by the extravascular blood and the cell-free serum obtained from it.

3. This property is of such a stable nature that it is also effective in the bodies of other animals, so that we are in a position, by means of the transfer of blood serum, to accomplish noteworthy therapeutic results.

[a] Rosenbach: Zur Aetiologie des Wundstarrkrampfes beim Menschen. Arch. f. klin. Chir., Berl., vol. 34, p. 306–317.

[b] Kitasato, S.: Ueber den Tetanusbacillen. Zeit. f. Hyg., vol. 7, 1889, p. 225.

[c] Kitasato, S.: Experimentelle Untersuchungen über das Tetanusgift. Zeit. f. Hyg., vol. 10, 1891, p. 304.

[d] Behring, E., and Kitasato, S.: Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei Theiren. Deut. med. Woch., vol. 16, 1890, p. 1113–1114.

4. The property of destroying the tetanus poison is absent in the blood of animals which have not been immunized against tetanus. If the tetanus poison be given to susceptible animals it may be demonstrated in the blood and other body fluids after the death of the animal.

For instance: A rabbit was immunized against tetanus to such a high degree of immunity that it was able to withstand 10 c. c. of a virulent culture containing the tetanus organism, 0.5 c. c. of which was sufficient to kill a normal rabbit. This rabbit was not only able to withstand the infection of living tetanus bacilli, but was also immune to the tetanus poison. Thus it withstood without any symptoms twenty times the quantity of tetanus poison sufficient to kill a normal rabbit.

This rabbit was bled from the carotid, and before the blood clotted 0.2 c. c. was given to a mouse and 0.5 c. c. to another. After twenty-four hours both of these treated mice and two control mice were inoculated with a virulent tetanus bacillus. The amount injected was so great that the control animals showed tetanus in twenty hours and were dead in thirty-six hours. Both treated mice, on the other hand, remained well.

The larger quantity of blood was allowed to stand until the serum separated. Of this serum six mice were injected with 0.2 c. c. each into the peritoneal cavity. Twenty-four hours later they were infected and remained well, while the control mice died in less than forty-eight hours.

It was further found that the serum had a therapeutic value in that the mice were first infected and afterwards protected by injecting the serum into the peritoneal cavity.

They further made experiments to demonstrate that the serum has an enormous power of destroying the poison.

Of a tetanus culture ten days old which was freed from bacilli by filtration, 0.00005 c. c. was sufficient to kill a mouse in four to six days and 0.0001 c. c. was sufficient to kill a mouse with certainty in two days. However, 5 c. c. of the serum from the tetanus immune rabbit was mixed with 1 c. c. of this culture, and the serum was allowed to act twenty-four hours upon the culture containing the tetanus poison. Of this mixture, four mice were given each 0.2 c. c., containing 0.0033 c. c. of the culture, or more than 300 times the dose otherwise fatal for mice. All four of the mice remained well. The control mice, on the other hand, died in thirty-six hours with 0.0001 c. c. of the fluid. The mice which were treated as above described, as well as those which had received the mixture of tetanus poison with serum, so far as can be told, remained well. Later they were injected repeatedly with virulent tetanus bacilli, which they withstood without a trace of symptoms.

This fact is of very particular note, because in the innumerable experiments no mouse, no rabbit, in fact no animal so far tested has shown a natural immunity against tetanus. We therefore may draw the conclusion that the above-mentioned explanation of the conditions of immunity which may be obtained promptly and without difficulty, and which may be accomplished without harm to the animals in the process of immunization, will prove of far-reaching value. It is to be understood that control animals were tested with the blood and serum of nonimmune rabbits. The same may be said to be true of cattle and sheep.

We permit ourselves from our results to venture the conclusion that, after finding a therapeutically potent substance useful for animals, it will also be useful in the treatment of diphtheria and tetanus in man. We only desire to call attention to one thing in conclusion: In former times the transfusion of blood was considered to be a heroic, but in certain cases a very powerful, curative agent. In later times it was thought that physiological salt solution was able to accomplish the same result. On the other hand, our experience makes memorial the phrase "Blut ist ein ganz besonderer saft."

Summary of Remaining Pages in Foregoing Paper

Prepared by Margaret Pittman

Stability of Tetanus Toxine (pp 16-18)

Tetanus toxine does not remain constant in solution. Success was obtained "by precipitating the filtered bouillon culture with ammonium sulphate, drying the precipitate over sulphuric acid, grinding to impalpable powder, and preserving it in vacuo under the influence of pentaphosphoric acid—a very strong dehydrating substance—in a cold, dark place." The dried toxine shows no loss for at least 25 months.

The Stability of the Standard Tetanus Toxine (PP 18-24)

The dried standard toxine (No. A) showed marked stability after exposure to sunlight for one and one-half hours, to 37°C for several days, to room temperature for greater than 11 days, etc.

The pioneer investigators reported destruction of the fluid toxine at 65°C and susceptibility to mineral acids, alkalis and salts; agar culture is the most poisonous; chickens, snakes, turtles and tritons are immune to toxin, formalin detoxifies the toxin. Anderson (Hyg. Lab. - Bull. No 39, 1907) reported the concentration of formalin exposure that rendered 100 MLD nonlethal for the guinea pig.

The Tetanus Toxine (pp 24-32)

An interpretative review of the published investigations on the toxine is given. Remarkable basic information has been reported. Some of the pertinent points covered were that (1) "tetanus toxine" is a complex substance—two toxins, tetanolysin and tetanospasmine; contains both a toxophore and a haptophore group; induces protective immunity—a free receptor in the blood combines with the toxophore and neutralizes the toxine; produces symptoms after a definite period of incubation, never less than 8 hours, and is inversely related to the size of the doses; one of the most poisonous of substances; susceptibility within animal species is remarkably constant but not between species—the MLD's for different species are given; harmless by mouth; and chemical nature is unknown. Remarks are made in regard to the best media to prodnce the toxine and the drying of toxine with the use of anmonium sulphate.

Mode of Action of Tetanus Toxine (pp 32-41)

Since ancient times it has been known that tetanus affects chiefly the nervous system. After a review of the investigations relating to neuronal action of the toxine it was stated "the weight of evidence is in favor of the view that the toxine produces its effect solely by the adsorption of the toxine by the nerves, whose axis cylinder substance has a specific affinity for the poison. They transport it to the cord and brain through axis cylinder substance itself."

The next three sections give the German, Italian, and French methods for Standardizing Tetanus Antitoxin (pp. 41-49).

The first two methods are very complex and are difficult to interpret. The strength of the French antitoxin is not stated on the label. "It is stated that the antitoxin dose of serum amounts to 1,000,000,000; that is, to protect a mouse against the smallest fatal dose it is sufficient to use 1/1,000,000,000 of its weight of serum."

The last section gives *Tables for diluting the Toxine and Antitoxin* (pp. 49-55).

"They are published not only because they will save time and the possibility of error incident to such calculations, but because it is of some importance that the same methods of dilution be used by different laboratories in order that the results may be strictly comparable."

The Regulation of Biologic Products, 1902-1972

Margaret Pittman

The acorn planted a century ago, when the Laboratory of Hygiene was established, has grown into an astounding tree. One of the spreading branches, the Regulation of Biological Products, has been of key importance in protecting the lives of millions of Americans. This article covers briefly the developments in biologic products at the National Institutes of Health from 1903, when the 1902 Biologics Control Act became effective, until the transfer of Regulations of Biologic Products to the Food and Drug Administration in 1972.



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I. Background: The Biologics Control Act of 1902

Biologics control in the United States followed pioneering research in Europe that opened the way to prevention and specific cure of infectious diseases by vaccination or treatment with antitoxins. In 1796 Jenner had introduced a vaccine against smallpox, and, by the late nineteenth century, Pasteur, Koch, Ehrlich, von Behring, Roux, and others were making progress against other diseases. In 1894 von Behring and Roux reported that diphtheria antitoxin could cure diphtheria. Very shortly, laboratories across Europe and the United States sent researchers to learn this technique. In 1894-95[1] New York City Laboratories began producing the antitoxin, using the unique strain of *Corynebacterium diphtheria* isolated by Anna Williams ("Park 8"). Production was also started at the Hygienic Laboratory after Kinyoun visited the Koch and Roux laboratories in 1894.[2] The antitoxin was being produced also by other laboratories. Sometimes it worked, other times not at all.

There were no requirements for potency or safety. A tragedy in 1901 provided the stimulus for Congressional passage of the Biologics Control Act in 1902. The city of St. Louis had engaged a bacteriologist to prepare diphtheria antitoxin for free distribution to physicians. In 1901, during a serious epidemic of diphtheria, ten children treated with antitoxin died of tetanus—not diphtheria. The horse from which the antitoxin was obtained had contracted tetanus. In the rush to provide the needed antitoxin, no safety test had been performed.[3] This tragedy convinced Congress and the public that producing antitoxin or vaccine was not a simple matter like weighing out a dose of a drug on a scale.

Under the provisions of the 1902 Biologics Control Act, which is reproduced in Appendix A, enforcement was delegated to the Division of Pathology and Bacteriology of the Hygienic Laboratory. Thus began the work of regulating biologicals.[4] In 1937 the work was placed in a newly established Division of Biologics Control. In 1944 this division was redesignated the Laboratory of Biologics Control, the name under which in 1948 it became a part of the National Microbiological Institute. In 1955 it was given separate division status within NIH and renamed the Division of Biologics Standards. In August 1972 the regulation of biologics control was transferred to the Food and Drug Administration.[5] The Chiefs or Directors of the control activities were Milton J. Rosenau, 1903-1909; John F. Anderson, 1909-1915; George W. McCoy, 1915-1937; Walter T. Harrison, 1937-1940; Milton V. Veldee 1940-1949; William G. Workman, 1949-1955; and Roderick Murray, 1955-1972.

High tribute is due to these directors for their sagacious handling of the development of regulations from the beginning, when the state-of-the-art in microbiology and immunology was in its infancy. This tradition has been continued through the complexity of controls of viral vaccines and their clinical evaluation. Praise also is due to the dedicated scientists and technicians without whom, the work could not have been accomplished.

II. General Aspects

Responsibility

The organization designated to regulate the control of biologics is responsible for establishing and maintaining standards of safety, purity, and potency of all biologicals as stated in the Biologics Control Act. The standards are set forth in prescribed regulations. "To apply our present knowledge to the improvement of biologics now on the market, to find better ways of producing and testing these biologics, and to help in the development of new immunizing agents against the infectious diseases that, so far, have baffled science - these are the ultimate aims of those concerned with the control of biologics," wrote Roderick Murray, Director of DBS in 1955.[6]

Development of Standards

Two forms of licenses are required: for establishment to sell a product and for each product sold. Licenses are issued only upon showing that the prescribed standards for safety, purity, and potency have been met. To ensure compliance with Public Health Service Regulations, specially trained members of the scientific staff inspect establishments applying for licenses. Those already licensed are inspected annually.

Specific requirements for a particular product are determined by the nature of the product and the state of knowledge about it. Two of the products first licensed—smallpox vaccine and diphtheria antitoxin, provide examples of this. Standards for smallpox vaccine initially emphasized bacterial content on the ivory points (by colony count) and the use of 50% glycerin to prevent growth of non-spore forming bacteria, yeasts, and molds;[7] animal test for freedom from tetanus spores; and a limit on the dating period of no more than three months "if stored at 5°C."[8] Close vigilance over the vaccine was demonstrated by the quick action taken when foot-and-mouth disease appeared in the calves used by two producers of smallpox vaccine: their licenses were revoked and their products condemned and destroyed. No human infection occurred.[2] For diphtheria antitoxin, requirements specified a sterility test for bacterial contamination, animal tests for safety (preservative, tetanus spores or other obnoxious poisons), and potency assay that ensured a specified number of immunity units per dose.[9]

Early requirements were promulgated on the basis of knowledge gained from the pioneer European investigators, often modified by research at the Hygienic Laboratory. The U.S. official immunity unit of diphtheria antitoxin, for example, was based on Ehrlich's normal serum unit with improvements in the precision of the assay.[9] The safety, purity, and potency tests were similar to those prescribed in the German official method for diphtheria antitoxins. The standardization of tetanus toxin was based on the American unit which was different from the German, Italian, and French units.[10] It has remained *the* standard unit.

Technical methods for evaluating a product have likewise depended upon its nature. Except for smallpox and rabies vaccines, classical bacteriological methods were followed until 1941 when new techniques were required for typhus vaccine, which was produced with rickettsiae propagated in the embryonated hen's egg. In the early 1950s the virus for poliomyelitis vaccine was propagated in animal tissue cultures. This procedure caused revolutionary changes to insure absence of adventitious agents that might be present in the animal tissue. For development and application of the new tests, a considerable expansion in the number of skilled research scientists and in space was required. It was at this time that the Division of Biologics Standards was established.



Dr. Pittman, with assistant, in 1930's DBC laboratory.

Research

From the beginning, research has been vital in the development of standards. Members of the Division have constantly worked to improve existing biologic products and to develop standards for new provisional products. In order that biologic standards be uniform throughout the world, these scientists serve on World Health Organization expert committees involved in the establishment of international standards, and they cooperate with scientists worldwide. Through their research, significant contributions have been made to medical science.

III. Biologic Products

Chronology of Product Licenses

The chronology of product licensing clearly reflects the state-of-the-art in microbiology, immunology, and the use of biologics in the practice of medicine. In 1903 four product licenses were issued—smallpox vaccine (6 U.S., 2 foreign) diphtheria antitoxin (6 U.S.), anti-streptococcus serum (1 U.S., 1 foreign), and toxins (1 U.S.) before required designation of specific toxin in the license. Between 1904 and 1906 only antibacterial sera were licensed. By the end of 1907 nine bacterial species were represented: dysentery, gonococcus, meningococcus, plague, pneumococcus, staphylococcus, streptococcus,

typhoid, and tubercle bacilli. A few more were added later. There also was thyroidectomized goat serum and horse serum. In 1907, anti-tetanic serum,—later changed to tetanus antitoxin, (3 U.S., 4 foreign) and a second viral vaccine, rabies (1 U.S.), were licensed. Later, additional manufacturers were licensed for rabies vaccine. The year 1907 was a turning point: manufacturers changed from producing "anti-bacterial" serum products exclusively to include "anti-bacterial" vaccines. Although most of the individual anti-bacterial sera were produced by only one manufacturer, many manufacturers produced the same bacterial vaccine. For example, thirteen companies were licensed for the "Colon-Bacillus" vaccine, and ten each for *Staphylococcus albus* and typhoid, and even seven for the nonpathogen *Micrococcus catharrhalis*. Some 30 bacterial species, representing practically all of those associated with bacterial diseases, had been included by the 1930s when the sulfa drugs, followed by the antibiotics, were introduced. One shortcoming of control during this period was that potency tests relative to efficacy had not been developed.

Beginning in 1917 licenses were issued for immunizing toxin products, for additional kinds of antitoxins, and for human bacterial and viral antisera. The toxin products included diphtheria toxin mixed with antitoxin (1917), diphtheria toxoid (1926), tetanus toxoid (1933), and scarlet fever streptococcus toxin (1925). Different combinations of diphtheria and tetanus toxoids with pertussis vaccine began in the 1940s, a period when the use of aluminum absorbed vaccines also became prominent. The antitoxins included botulinus (1921) perfringen and other gas gangrene species (1931-1939), scarlet fever streptococcus (1925) and gonococcus (1937). The human antisera included pertussis, poliomyelitis and mumps (1939-1941).

In 1941 the first rickettsial vaccine, typhus, produced by growing the microorganism in embryonated hen's egg, was quickly followed by other rickettsial and viral vaccines—influenza (1945), mumps (1950), and yellow fever (1953), or tissue cell culture from embryos for measles (1963), and rubella (1969). Viral vaccines produced from virus grown in propagated animal tissue culture were poliomyelitis vaccine (1955) and adenovirus (1957). Other viral vaccines included equine encephalitis (eastern and western) and Coxsackie 21. Bacterial vaccines included BCG (1950), anthrax (1970) and a number of bacterial vaccines for allergen extracts.

During World War II blood and blood-derivatives were licensed, including whole blood for transfusion, plasma, albumin, and other derivatives. Since 1972, when the functions of DBS were transferred to FDA,[5] licenses have been issued for four bacterial capsular polysaccharides—the meningococcus Groups A and C, pneumococcus-23 valent, and *Hemophilus influenzae* type b. In addition, hepatitis B antibody for screening bloods, for transfusion and derivatives, for the presence of hepatitis B antigen has been licensed.[11]

Classification of Licenses

By 1972, 450 establishment licenses had been issued but only 235 (16 of these were foreign) were active.[12] Product licenses numbered around 1300, covering approximately 300 specific products that could be classified as allergenic extract, antisera, antitoxins, bacterial vaccines, toxins, toxoids, viral and rickettsial vaccines, blood and blood derivatives, immune serum globulins, diagnostic substances for skin tests, venoms, and antivenoms.

IV. Selected Contributions

Allergenic Products

Allergenic products are administered to man for the diagnosis, prevention or treatment of allergies. Sources of allergens are numerous, and identification of the specific antigen has progressed slowly. In 1960, DBS initiated the development of standards under the direction of Harold Baer, Chief, Laboratory of Allergenic Products. General Additional Standards for all allergenic products with specific standards for only one product, short ragweed pollen extract, have been prescribed.[11] Descriptions of this product,[13] of fire ant venom protein,[14] and of poisonous *Ancardiaceae* (e.g., poison ivy),[15] illustrate different kinds of reactive substances. The technic for performing the skin test, prepared by a committee on standardization of which Baer is a member,[16] has contributed significantly to the evaluation of results.

Antiserum

Meningococcus. Deficiency in type-specific antibodies was one cause of ineffectiveness of some bacterial antisera. One was antimeningococcus serum. In the early 1930s there was a massive epidemic of meningitis. The antiserum formerly thought to be efficacious was largely ineffective. NIH invited Sara E. Branham to investigate the cause. In her 1956 review, Dr. Branham described the two early classification of the meningococcus and the final group-specific classification.[17] Encapsulation of the organism was discovered. Type IIα was identified as Group C. Using Petrie's observation that a precipitin halo develops around a colony on immune serum agar plates, group (type) specificity was shown.[18] A recommended mouse protection test likewise showed specificity.[19] Mouse assayed potency and plate precipitation of Group I antibodies showed high correlation.[20] By the early 1940s, however, treatment with meningococcus antiserum was superceded by treatment with sulfonamides.

Antitoxin

The precise methods used to develop standards for diphtheria antitoxin by Rosenau[9] and for tetanus antitoxin by Rosenau and Anderson[10] provided a model for the development of other products. Following the standard model for diphtheria antitoxin, for example, Ida A. Bengtson establisbed the immunity units for botulinus antitoxins, A, B, and C. Previously the unitage ranged from 2 to 450 per ml.[22] Bengtson also established the standards for the gas gangrene antitoxins—*Clostridium histolyticum*,[23] *C. perfringens*,[24a] *C. septicum*,[24b] *C. oedematiens*,[25] and *C. sordelli*.[26] She also supplied the first international standard preparations—the respective toxins and antitoxins—to the League of Nations Committee in Copenhagen, Denmark.[27] Although the United States was not a member of the League, George W.

McCoy, Director of NIH, served as an invited unofficial member of the Committee.

In addition to the development of standard units, Rosenau and Anderson investigated the phenomenon of sensitivity to horse serum following antitoxin treatment. Their research on such reactions, which caused concern about the safety of antitoxins, provided pioneer contributions in the study of anaphylaxis.[21]

Bacterial Vaccines

Efficacy of only a few of the many bacterial vaccines licensed before the advent of antibacterial drugs was encouraging but variable. Those containing a toxin (toxoid) or capsular substance proved to be the most effective. A brief discussion of the cholera, pertussis, and typhoid vaccines will illustrate both successes and difficulties.

Cholera Vaccine. The first pathogenic bacterium isolated by the Hygienic Laboratory was *Vibrio cholerae*, successfully obtained in 1887 from an immigrant on a ship in New York Harbor. In the 1960s, during the eighth pandemic of cholera, the World Health Organization (WHO) and the newly established Pakistan-SEATO Cholera Research Laboratory in Dacca, East Pakistan (Bangladesh) stimulated work on a potency test for cholera. This potency assay[28] correlated with human efficacy of the vaccine,[29] although of relatively short duration. This SEATO program was organized by Joseph E. Smadel, then Associate Director of NIH (1956-60). From 1960 to his death in 1963 he was Chief of the Laboratory of Virology and Rickettsology, DBS

Later it was shown that pathogenesis and immunity of cholera is effected by a cholera toxin. Studies continue elsewhere on how to provide prolonged immunity against cholera.

Pertussis Vaccine. Pertussis vaccine regulation was concerned with (i) control of the bacterial content; (ii) development of a potency assay, (iii) standardization of potency unitage; (iv) relation of unitage to protection against pertussis, (v) toxic substances of *Bordetella pertussis*, (vi) the concept that pertussis is toxin mediated, (vii) an animal model, and (viii) neurotoxicity of *B. pertussis*.

In order to obtain uniformity in the bacterial content of pertussis vaccines, an opacity standard was prepared with fine pyrex glass particles. It was equivalent to 10 X 10^9 pertussis bacteria, in aged vaccine, as determined by direct count. In comparison with the standard, the average of lots per manufacturer differed from 0.4 to 1.6 fold.[30]

For many years attempts to develop a potency assay for pertussis vaccine had failed. The first successful potency assay for pertussis vaccine was developed as a consequence of John F. Norton's observation that the vaccine protected against intracerebral challenge of *B. pertussis*. The original potency requirement was based on relative potency of the bacteria in the vaccine and in the reference (1949). In 1953, a value of 12 units per total immunizing dose was prescribed. Pittman[31] described the development of the potency assay, standardization, and the rapid drop in mortality of children that followed. In the collaborative assessment of the vaccines in British field trials, it was shown that 12 units (three injections) afford about 85% protection against home exposure,[32] with a high correlation between unitage and protection against home exposure. The World Health Organization adopted the criteria that a single dose should contain no fewer than 4 units.

B. pertussis induces a diversity of pathophysiological reactions. Although the toxic factor(s) had not been identified, a mouse toxicity test was prescribed in the first requirements for pertussis vaccine. Pittman and Cox[33] reported on analysis of the results of 9.5 years of testing. Some years later Pittman proposed that both the harmful effects of pertussis and its prolonged immunity were caused by an exotoxin.[34] Study of experimental respiratory infection in the mouse emphasized the disparate pathophysiological responses,[35] and this was followed by the elucidation of pertussis as a toxin-mediated disease.[36] Finally, accrued evidence suggested that encephalopathy is caused by neuronal necrosis effected by a toxin-excited neurotransmitter, such as glutamate or aspartate.[37]

Typhoid Vaccine. Evaluation of the potency of typhoid vaccine has been complicated by the lack of a susceptible animal. However, with the use of the so-called N ip assay it was confirmed that Vi antigen is a major protective antigen and that the relative potency of the vaccines reflected efficacy.[38] The results of the laboratory assay of WHO field trial vaccines, which included the reference NIH 4, prompted the selection of a Vi containing vaccine as the U.S. standard and promulgation of Additional Standards for Typhoid Vaccine. Later Wong *et al.*[39] by enzymatic removal of capsule confirmed and emphasized the importance of the Vi antigen.

Invasion of the blood by salmonellae is limited to *Salmonella typhi* and only this species has a capsule (Vi polysaccharide). Infection and purified Vi stimulate a rise in Vi antibody. It is reasonable to consider that Vi antigen alone or conjugated to a protein may provide immunity and thereby eliminate the undesirable pyrogenic reaction after whole cell vaccination.

Mycobacterial and Fungal Antigens

Many questions about various aspects of tuberculosis remain unanswered. Sotiros D. Chaparas, Chief and his colleagues in the Laboratory of Microbacteria and Cellular Immunology demonstrated the correlation of *in vitro* cellular transformation and skin reactivity of mycobacterial antigens.[40,41] Chaparas, Fuller and Seligmann investigated methods for sensitization of guinea pigs in the control testing of fungal antigens.[42] Additional Standards for BCG Vaccine and Tuberculin have been prescribed.[11]

Mycoplasma

Mycoplasma are frequent contaminants of continuous cell lines and, less frequently, of primary cell cultures.[43] In 1962 Michael F. Barile, Chief of the Laboratory of Mycoplasma, developed the definitive test for Mycoplasma, required during the manufacture of viral vaccines.[11] Barile *et al.* have identified more than 1000

mycoplasma from contaminates in cell cultures, and determined their source.[44] After it was shown that *Mycoplasma pneumoniae* was a primary cause of atypical pneumonia and that vaccination against the disease appeared encouraging, Barile and colleagues developed a hamster potency assay for the evaluation of *M. pneumoniae* vaccine.[45]

Toxin

M. F. Barile directed the investigation for a suitable toxin to be used as the U. S. Standard for the Schick Test. The guinea pig erythema potency assay, established as the officially prescribed assay, is significantly more reproductive than the minimum lethal dose method.[46]

Toxoid

The effects of adjuvant tetanus toxoids on the duration of the protective level of antitoxin (0.01 u/ml) against neonatal tetanus in New Guinea, were studied by R. MacLennan, F. D. Schofield, and DBS colleagues. Results showed that oil-adjuvant toxoid (one injection) and $AlPO_4$ toxoid (two injections) protected against neonatal tetanus. The average protective antibody level persisted for more than 3 yrs, but fewer than 4 years. Plain toxoid (three injections) was protective, but duration was less than 1 year. Unacceptable side-effects were induced by subsequent lots of oil-adjuvant toxoid, however, and this has precluded the use of this adjuvant.[47a,b,c,d,e]

Viral Vaccines

Smallpox Vaccine. The first vaccine against any infectious disease, smallpox vaccine, was also the first licensed vaccine in the United States (1903). A few years ago this once prevalent and dreaded disease was declared by WHO to be eradicated. The Laboratory of Control Activities contributed to eradication by establishing standards for dried, potent, stable vaccine that was used to vaccinate large populations. Potency was standardized as the number of pocks produced on the chorioallantoic membrane of chick embryos relative to the U. S. reference vaccine. This method, proposed by Fuller and Kolb,[48] replaced the rabbit scarification method. Hornibrook and Gebbard[49] first showed that smallpox vaccine could be dried without loss of potency.

Rabies Vaccine. Rabies vaccine was first produced in the United States by a few widely scattered laboratories known as "Pasteur Institutes." Exposed persons often travelled several hundred miles to be treated at these laboratories. In 1906, the Hygienic Laboratory resumed study of the vaccine, and by 1908 it was treating persons in the Washington, D.C. area. Shortly thereafter, the Laboratory began shipping rabbit spinal cord preserved in glycerin to state health officials who had laboratory facilities for treatment. This practice was discontinued in 1921, after several commercial manufacturers of biologics had developed facilities for treatment.[2] Later advances included desiccation of the cord, in vacuo, over P_2O_5, after which it was preserved with glycerin. Further progress was made by achieving inactivation with UV, a replacement for phenol. To eliminate the infrequent neurological reaction caused by the rabbit brain tissue, the virus was propagated in embryonated duck egg. Presently, the preferred method is propagation of the virus in cell culture. In 1948 Habel and Wright developed a mouse test for potency assay.[50] Since 1953, the modified Seligmann test which is the NIH test has been used. A comparison of the NIH test with an antibody-binding test by Fitzgerald, *et al.*, suggests promise for an *in vitro* assay test.[51]

Other Viral Vaccines

Among the challenging problems encountered in standardizing live viral vaccines propagated in animal cell culture was the detection of adventitious agents in mammalian or poultry embryo tissue cells.[52] In 1962, for example, Eddy *et al.* identified simian virus 40 in rhesus monkey cell cultures.[53] For live oral poliovirus vaccine, a monkey neurovirulence test was developed by Ruth L. Kirschstein and colleagues to provide assurance that seed viral strains and the vaccine were not neurovirulent.[54,55]

Trials of a live measles virus vaccine in Upper Volta by Meyer *et al.* showed the feasibility of combining live measles, smallpox and yellow fever vaccines and their administration by jet inoculation.[56] As many as 300 injections could be made in one hour; approximately 730,000 children were vaccinated.

Three articles in the 1960s from the Laboratory of Viral Immunology described remarkable progress in protecting against the so-called German measles, from the attenuation of the rubella virus to the standardization of a live rubella vaccine. Parkman *et al.*[57] described its development and laboratory characterization. Myer *et al.*[58] reported on production and clinical trials, and Stewart *et al.*[59] described the development of the simple, inexpensive rubella-virus hemagglutination test that is used effectively for diagnosis and for determining immunity status. Its use in preventing of disease and congenital defects has been very successful.

Influenza vaccine was one of the first viral vaccines in which the virus was propagated in hen embryos. Bernice E. Eddy developed the first potency assay.[60] The hemagglutinating and neuraminidase antigens that determine the specificity of the strains tend to change, hence the formula for the vaccine is assessed yearly.

Rickettsial Vaccines

The microorganisms that cause typhus, Rocky Mountain spotted fever and other rickettsial diseases are propagated in embryonated hen's egg for vaccine production. Typhus vaccine was the first to have extensive use. Transmitted by lice, typhus caused devastating epidemics that ravaged southern Europe during World War I and the years immediately following. During World War II, every serviceman in North Africa and Italy (and many liberated civilians) were vaccinated against typhus. Among U. S. military personnel there were only 64 cases of typhus and no deaths.

In 1941 Bengtson and Topping used rickettsial antigens to develop a complement fixation test that specifically identified antibodies of epidemic typhus fever.[61]

Blood and Blood Derivatives

The first program on blood and blood products was developed during World War II by Roderick Murray and J. T. Tripp. They established standards and supervised plasma production in commercial establishments from blood collected by the American Red Cross. Some 13,000,000 pints of blood were processed to plasma and albumin.

A most disturbing development was the presence of an icterogenic agent in some pools of plasma. In 1942, 28,000 servicemen injected with yellow fever vaccine prepared with human serum as a stabilizing agent fell ill with jaundice, and 100 died. Murray *et al.* confirmed that post-transfusion hepatitis was caused by whole blood from carriers of hepatitis.[62,63] Currently, the risk of transmission of serum hepatitis is practically nil. All bloods for transfusion or for preparation of other products are screened for presence of hepatitis B antigen with use of "Antibody Hepatitis B Surface Antigen."

There are 34 whole blood products and 45 diagnostic substances, 41 of which are for blood grouping and typing. Among blood products are four human serum globulins: Immune Globulin for general prophylactic use and Measles, Mumps and Tetanus Immune Globulins. Of the approximately 5,000 blood banks in the country, at least 250 operate under federal license and are inspected each year.

In addition to preventing hepatitis, research in the blood program has continued to be vital. When, for example the military planned to stock pile large amounts of albumin in caves for emergency use, Finlayson *et al.* developed assays to detect the effect of long-term storage on the albumin.[64,65]

Aronson made fundamental observations on the proteolytic nature of the conversion of human prothrombin to thrombin.[66,67]

Coral Snake Venoms and Antivenin

About 50 species of coral snakes inhabit southern North America, Central America, and South America. Their neurotoxic and hemolytic venoms may be fatal to man, hence antivenom is needed. Cohen, Berkeley, and Seligmann, studied the serological relationship between six coral snake venoms from North, Central, and South America.[68] *Micrurus frontalis* venom, which elicited the most cross-reactive neutralizing antibody of the venoms tested, would be the most suitable for the production of polyvalent coral snake antivenin. Two other antivenoms are licensed—*Crotalidae* polyvalent and *Latrodectus mactans*.

V. General Standards for Biologics

Sterility Testing

Sterility testing is essential to assure freedom from bacterial contamination in biological products. At the beginning, the culture medium was dextrose meat infusion broth that was used in the Smith fermentation tube and temperature of incubation was 37°C.[69]

In the early 1940s, Brewer or Linden fluid thyoglycollate medium was prescribed. It promoted aerobic and anaerobic growth and neutralized the inhibitory action of mercurial preservatives. Reports that the Eh indicator, methylene blue, inhibited growth of a few contaminants prompted an investigation of growth promoting activity of each ingredient in the media. A large number of bacterial contaminants and two temperatures of incubation, 37°C and 25°C, were used. Resazurin replaced methylene blue. With one slight change, reduction in *l-cystine* from 0.75 g to 0.5 g/liter, the medium developed in 1946[70] has remained one of the official media for sterility testing. It was adopted by the U.S. Pharmacopeia and internationally by the World Health Organization.

Another important change was the specification of a lower incubation temperature of 30° - 32°C or the use of two temperatures 37°C and 25°C. The reason for the revision was that some bacteria isolated from whole blood or plasma that had caused a severe reaction, at times fatal, would not grow at the incubation temperature of 37°C but would grow at 4°C.[71]

Pyrogenicity Testing

In the early 1940s, the increasing use of intravenous therapy, particularly blood, plasma and other blood products, drew attention to the role of bacterial contaminants as the cause of fever reactions (pyrogenicity). Probey and Pittman studied the pyrogenicity of 28 bacteria isolated from plasma.[72] All were capable of inducing fever in rabbits, but reaction varied both quantitatively and qualitatively. This study, which also showed that growth in serum enhanced pyrogenicity and that the number of bacteria should not exceed 5000 per ml. before filtration to avoid pyrogenicity, contributed to the promulgation of the rabbit pyrogen test.

The Limulus test for endotoxin (pyrogen), shown by Cooper *et al.* to be satisfactory for radiopharmaceuticals and biologics[73] was adopted officially.[11] Endotoxin affects coagulation of the lysate of amebocytes of the horseshoe crab, *Limulus polyphemus*.

Methods

Freeze-Drying. About 1937, NIH purchased its first equipment for freeze-drying bacterial cultures. This method has been invaluable for references and standards in the control of biologics, also for certain products. Seligmann and Farber further enhanced its usefulness by examining the influence of residual moisture and recommending techniques.[74]

Mice. To obtain reproducible results in mouse potency assays of vaccines, the mouse strain used is very important. Lajos Csizmas showed a correlation between the protective response to tetanus toxoid and pertussis-histamine sensitizability (HS) of the mouse strain; Pittman reported similar results for pertussis vaccine.[75] The mouse HSFS/N line strain was selectively bred for high HS from the N:NIH(SW) strain by Manclark *et al.*[76]

Safety (oncogenicity)

1. *Biologics showed no evidence of oncogenicity.* In the 1960s, oncogenic screening of biologic products was initiated under NIH contracts to DBS. All bacterial and viral vaccines, toxins, toxoids, and venoms as well as the

seven preservatives used in these products were tested under subcontracts for oncogenicity in animals. The results were negative.

2. *Mineral oil adjuvants may be hazardous.* In 1966, DBS initiated a series of studies under contract to evaluate the safety of mineral oil adjuvants. At that time, there was much interest in use of oil adjuvants in allergens and other products. Untoward reactivity of tetanus toxoid containing mineral oil had been reported in 1965 by MacLennan *et al.*[47a] The 1972 final report by Murray, Cohn, and Hardegree states "these data suggest that the use of mineral oil adjuvants in human populations may be hazardous and that they should not be recommended for general use in man."[77]

IV. Summary

The unfolding of advancements in knowledge has made possible the development of biologics products (and their regulation) that are applicable to the prevention, treatment, or cure of diseases or injuries of man. Gradually there was understanding of the specific immunizing antigens of vaccines and their relation to human efficacy, and of the groups and types of blood that make possible treatment with blood and blood products.

Benefits are exemplified by the control of infectious diseases by routine vaccination with toxoid and viral vaccines, also other vaccines as indicated, and life saving treatment with blood expanders: whole blood and blood derivative. Millions and millions of lives have been saved.

The purpose of the Public Health Service is to contribute to public health. The Regulations of Biological Products has served well.[78]

The outstanding progress was made possible not only by in-house scientists but by cooperation of scientists worldwide and of industry, all in a research context.

It is not possible to recognize all of the contributors. Omission does not diminish in any way their contributions.

Appendix A

Excerpts from the Public Health Service Act,
As Amended
Title III - General Powers and Duties of the Public Health Service
Part F - Biological Products
Regulation of Biological Products
42 U.S.C. 262

Sec. 351. (a) No person shall sell, barter, or exchange, or offer for sale, barter, or exchange in the District of Columbia, or send, carry, or bring for sale, barter or exchange from any State or possession into any other State or possession or into any foreign country, or from any foreign country into any State or possession, any virus, therapeutic serum, toxin, antitoxin, or analogous product, or arsphenamine or its derivatives (or any other trivalent organic arsenic compound), applicable to the prevention, treatment, or cure of diseases or injuries of man, unless (1) such virus, serum, toxin, antitoxin, or other product has been propagated or manufactured and prepared at an establishment holding an unsuspended and unrevoked license, and prepare such virus, serum, toxin, antitoxin, or other product for sale in the District of Columbia, or for sending, bringing, or carrying from place to place aforesaid; and (2) each package of such virus, serum, toxin, antitoxin, or other product is plainly marked with the proper name of the article contained therein, the name, address, and license number of the manufacturer, and the date beyond which the contents cannot be expected beyond reasonable doubt to yield their specific results. The suspension or revocation of any licenses shall not prevent the sale, barter, or exchange of any virus, serum, toxin, antitoxin, or the product aforesaid which has been sold and delivered by the licensee prior to such suspension or revocation, unless the owner or custodian of such virus, serum, toxin, antitoxin, or other product aforesaid has been notified by the Secretary not to sell, barter or exchange the same.

(b) No person shall falsely label or mark any package or container of any virus, serum, toxin, antitoxin, or other product aforesaid; nor alter any label or mark on any package or container of any virus, serum, toxin, antitoxin, or other product aforesaid so as to falsify such label or mark.

(c) Any officer, agent, or employee of the Department of Health, Education, and Welfare, authorized by the Secretary for the purpose, may during all reasonable hours enter and inspect any establishment for the propagation or manufacture and preparation of any virus, serum, toxin, antitoxin, or other product aforesaid for sale, barter, or exchange in the District of Columbia, or to be sent, carried, or brought from any State or possession into any other state or possession or into any foreign country, or from any foreign country into any State of possession.

(d) Licenses for the maintenance of establishments for the propagation or manufacture and preparation of products described in subsection (1) of this section may be issued only upon a showing that the establishment and the products for which a license is desired meet standards, designed to insure the continued safety, purity, and potency of such products, prescribed in regulations, and licenses for new products may be issued only upon a showing that they meet such standards. All such licenses shall be issued for the maintenance of establishments for the propagation or manufacture and preparation, in any foreign country, or any sucb products for sale, barter, or exchange in any State or possession shall be issued upon condition that the licensees will permit the inspection of their establishments in accordance with subsection (c) of this section.

(e) No person shall interfere with any officer, agent, or employee of the Service in the performance of any duty imposed upon him by this section or by regulations made by authority thereof.

(f) Any person who shall violate, or aid or abet in violating, any of the provisions of this section shall be punished upon conviction by a fine not exceeding $500 or by imprisonment not exceeding one year, or by both such fine and imprisonment, in the discretion of the court.

(g) Nothing contained in this Act shall be construed as in any way affecting modifying, repealing, or superseding the provisions of the Federal Food, Drug, and Cosmetic Act (U.S.C., 1940 edition, Title 21, ch.9).

Preparation of Biological Products
42 U.S.C. 263

Sec. 352. (a) The Service may prepare for its own use any product described in section 351 and any product necessary to carrying out any of the purposes of section 301.

(b) The service may prepare any product described in section 351 for the use of other Federal departments or agencies, and public or private agencies and individuals engaged in work in the field of medicine when such product is not available from establishments licensed under such section.

Margaret Pittman, Ph.D.

Dr. Margaret Pittman was born in 1901 in Prairie Grove, Arkansas. Following her graduation from Hendrix College with an A.B. degree in 1923, she earned an M.S. and Ph.D. in Bacteriology from the University of Chicago in 1929. Before coming to NIH she worked at the Rockefeller Institute from 1928 to 1934. From 1936 until 1971 she was a Microbiologist at NIH, in NMI and DBS. In 1957 she became Chief of the Laboratory of Bacterial Products—one of the first women to become Chief of an NIH laboratory. While at NIH Dr. Pittman has been recognized and honored for her research contributions in respiratory infections, meningitis and conjunctivitis and the standardization of bacterial vaccines and toxins, with emphasis on pertussis as a toxin mediated disease after she retired.

In 1971 she officially retired from Federal service, but she has continued as a Guest Worker in the Division of Biologic Standards and the FDA Center for Drugs and Biologics located at NIH.

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TREASURY DEPARTMENT.
Public Health and Marine-Hospital Service of the United States.
WALTER WYMAN, Surgeon-General.

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M. J. ROSENAU, Director.
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A STUDY OF THE CAUSE OF SUDDEN DEATH FOLLOWING THE INJECTION OF HORSE SERUM.

BY

M. J. ROSENAU
AND
JOHN F. ANDERSON.

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A STUDY OF THE CAUSE OF SUDDEN DEATH FOLLOWING THE INJECTION OF HORSE SERUM.

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It has long been known that the blood of certain animals is poisonous when transfused or injected into certain other species.

Many instances might be cited showing that the blood serum of one animal has poisonous properties when injected into an animal of another species. But the blood serum of the horse apparently lacks such poisonous action. Very large quantities of the blood serum of the horse may be injected into man, rabbits, guinea pigs, and many other animals without serious inconvenience, except occasionally a slight reaction at the site of inoculation.

In a certain proportion of cases the injection of horse serum into man is followed by urticarial eruptions, joint pains, fever, swelling of the lymph nodes, edema, and albuminuria. This reaction, which appears after an incubation period of eight to thirteen days, has been termed by Pirquet and Schick the "serum disease."

In exceptional instances sudden death bas followed an injection of horse serum in man.

These studies were taken up in October, 1905, in order to throw light upon the cause of this unfortunate accident. We have shown that ordinarily horse serum is a comparatively bland and harmless substance when injected into certain animals; but these animals may be rendered so susceptible that an injection of horse serum may produce sudden death or severe symptoms. For example, large quantities of horse serum may be injected subcutaneously or into the peritoneal cavity of a guinea pig without apparently causing the animal the least inconvenience. However, if a guinea pig is injected with a small quantity, say $\frac{1}{250}$ c. c., of horse serum and after the

expiration of a certain interval is again injected with horse serum the result will probably be fatal. The first injection of horse serum has sensitized the animal in such a way as to render it very susceptible to a toxic principle in horse serum. It is probable that when the guinea pig is injected with the first, or sensitizing, quantity of serum the strange proteid contained in the horse serum develops in the body of the guinea pig "antibodies" which, when brought into contact with more horse serum given at a second injection, produce either a union or a reaction, which causes the toxic action.

A certain time is necessary to elapse between the first and second injections of horse serum before this toxic action is able to manifest itself. This "period of incubation" is from ten to twelve days, and corresponds suggestively with the period of incubation of the serum disease which Pirquet and Schick place at eight to thirteen days.

Guinea pigs may be sensitized with exceedingly small quantities of horse serum. In most of our work we used quantities less than $\frac{1}{100}$ c. c. and we found in one instance that $\frac{1}{1,000,000}$ c. c. of horse serum was sufficient to render a guinea pig susceptible.

It also requires very small quantities of horse serum, when given in a second injection, to produce poisonous symptoms. One-tenth c. c. injected into the peritoneal cavity is sufficient to cause the death of a half-grown gninea pig. One-tenth c. c. of horse serum injected subcutaneously is sufficient to produce serious symptoms. The fact that this toxic action may be developed by such small quantities of serum and the fact that exceedingly small quantities are sufficient to prodnce symptoms and death upon a second injection, a priori places both the sensitizing and the toxic principle in the horse serum in the "haptin group" of substances in the sense used by Ehrlich.

A still further indication that the side-chain theory in its broadest sense may be applicable is the further fact that immunity may be produced against the toxic action by multiple injections of the serum.

While at first we thought that diphtheria antitoxin had some relation to this action, we are now able to state positively that it has nothing whatever to do with the poisonous action of horse serum; further, that diphtheria antitoxin in itself is absolutely harmless. The toxic action which we have studied is caused by a principle in normal horse sernm and is entirely independent of the antitoxic properties of the serum.

SUMMARY AND CONCLUSIONS.

Normal horse serum, when injected into the peritoneal cavity of a normal guinea pig, produces no symptoms. When injected subcutaneously there may result at most a slight local reaction consisting of swelling and edema, which gradually disappears.

Antitoxic horse serum is equally harmless for normal guinea pigs.

Horse serum is, however, poisonous to a guinea pig which has previously been injected with horse serum. The "period of incubation" or time necessary to elapse between the first and second injection is about ten days. Under these circumstances, horse serum is poisonous whether injected subcutaneously or into the peritoneal cavity.

The first injection of horse serum renders the guinea pig susceptible.

The symptoms caused by the injection of horse serum into a susceptible guinea pig are respiratory embarrassment, paralysis, and convulsions, followed by death. The symptoms come on usually within ten minutes after the injection, and when death results it usually occurs within one hour, frequently in less than thirty minutes, and sometimes within a few minutes.

The poisonous principle in horse serum appears to act upon the respiratory centers. The heart continues to beat long after respiration ceases.

The toxic action of horse serum bears no relation to diphtheria. The poison is not *toxone*. Guinea pigs can not be rendered susceptible by previous infections with the *B. diphtheriæ* or by previous injections with diphtheria toxine.

It seems from our work, however, that guinea pigs first injected with a mixture of diphtheria toxine plus horse serum are more sensitive to subsequent injections of horse serum than are guinea pigs sensitized with a first injection of horse serum alone.

DIPHTHERIA ANTITOXIN PLAYS NO PART IN THIS POISONOUS ACTION AND IN ITSELF IS HARMLESS.

As soon as we realized that the toxic principle in horse serum exerts its action in quantities so minnte as to place it almost in the category of the ferments and, further, when we concluded from our work that this toxic principle is doubtless one of those highly organized and complex proteid substances belonging to the "haptin group" in the sense used by Ehrlich, we recognized how futile it would be with present methods to attempt to isolate this substance.

Nevertheless we devoted much time and study to the relation of this toxic principle to various chemical, physical, and electrical influences. The practical importance of eliminating or neutralizing this toxic principle in horse serum is at once evident.

It is probable that when the strange proteid is introduced into the guinea pig it causes a reaction resulting in a production of "antibodies," so that when a second injection of horse serum is given there is probably either a union or a reaction between the antibodies and a substance in the horse serum which produces the poisonous effect.

This poisonous principle is quantitatively specific; that is, guinea pigs treated with horse serum are rendered somewhat susceptible to the subsequent injection of the serum of another animal. Guinea pigs treated with the serum of another animal are slightly sensitive to the toxic action of horse serum.

Guinea pigs treated with the serums of various animals and subsequently injected, are much more susceptible to homologous serums than to heterologous serums.

This poisonous action has no relation to hemolysis. Our work proves that blood serum may contain an acute poison entirely independent of any hemolytic action. Normal horse serum has no lytic power upon the red corpuscles of the normal guinea pig.

This poisonous action has no relation to the specific albuminous precipitins.

The poisonous principle in horse serum is not affected by a temperature of 60° C. for 6 hours, but it is destroyed at 100° C. for 15 minutes.

The poisonous principle is filterable through porcelain, is not injured by drying, and can not be separated by precipitation with ammonium sulphate and subsequent dialysis.

The following chemical substances do not oxidize, neutralize, or precipitate the poisonous principle in horse serum: Butyric acid, permanganate of potash, citrate of soda, alcohol, succinic peroxide acid (alphozone), hydrogen dioxide, and ammonium sulphate. The presence of chloroform or trikresol (0.4 per cent) does not interfere with this poisonous action.

Serums eight years old are as toxic as those freshly separated.

Exposure to X-rays does not affect the poisonous action of horse serum.

It requires about 10 days after the first injection of horse serum for a guinea pig to show susceptibility to a second injection. A guinea pig remains susceptible a very long time, at least 160 days.

As small a quantity as $\frac{1}{1,000,000}$ c. c. of horse serum was sufficient in one instance to render a guinea pig susceptible. Quantities

varying from $\frac{1}{250}$ to $\frac{1}{1000}$ c. c. almost invariably render guinea pigs highly susceptible when given in the toxine-antitoxine mixture.

One-tenth c. c. of horse serum injected into the peritoneal cavity of a susceptible guinea pig is sufficient to cause death. The same quantity inoculated subcutaneously may cause serious symptoms.

There is some evidence to show that the sensitizing substance in horse serum is the same as the poisonous substance. The sensitizing substance is not affected by precipitation with ammonium sulphate and dialysis.

Guinea pigs may he sensitized with horse serum that has been dried and redissolved.

The sensitizing substance is not affected by a temperature of 60° C. for 6 hours.

It is probable that small quantities of horse serum render a guinea pig more susceptible than do large quantities. If this be true, it is due, perhaps, to the fact that large quantities, owing to slow absorption or prolonged reaction, partly immunize the guinea pig at the same time that it is being sensitized.

The sensitizing substance apparently is not free in the blood serum of guinea pigs.

An active immunity against this toxic principle may readily be established by repeated injections of horse serum, at short intervals, into a guinea pig. Although guinea pigs may be immunized actively in this manner we have not yet succeeded in transferring this immunity in the blood serum or body juices to another guinea pig. It therefore appears that the immune bodies, if such exist against the toxic action of horse serum, are not free in the blood and body juices contrary to the case in diphtheria.

Guinea pigs may be sensitized to the toxic action of horse serum by feeding them with horse serum or horse meat.

The fact that guinea pigs may be rendered susceptible by the feeding of strange proteid matter opens an interesting question as to whether sensitive guinea pigs may also he poisoned by feeding with the same serum given after a proper interval of time. If man can be sensitized in a similar way by the eating of certain proteid substances may not this throw light upon those interesting and obscure cases in which the eating of fish, sea food, and other articles of diet habitually cause sudden and sometimes serious symptoms?

The susceptibility to the toxic action of horse serum is transmitted hereditarily from the mother guinea pig to her young.

These results upon the hereditary transmission of the susceptibility to the poisonous action of horse serum in guinea pigs may throw light upon the well-known hereditary tendency to tuberculosis in children born of a tuberculous parent. There are certain analogies between the action of tuberculosis and horse serum. Both

may produce a hypersusceptibility and also a certain degree of immunity. Now that we have proved that this hypersusceptibility or anaphylactic action in the case of horse serum may be transmitted hereditarily in guinea pigs, may it not throw light upon the fact that tuberculosis "runs in families?"

Demonstrations of the hereditary transmission of acquired characters are comparatively rare in biology. While there are several recorded instances demonstrating that immunity to certain infectious diseases may be transmitted from a mother to her young, yet, as far as we know, this is the first recorded instance in which hypersensitiveness, or anaphylaxis, has been experimentally shown to be transmitted from a mother to her young.

Other albuminous substances, such as skimmed milk, peptone, hemoglobin, egg albumin, and vegetable proteids possess no poisonous action upon guinea pigs sensitized with horse serum. Whether guinea pigs are rendered susceptible to a subsequent injection with the same albuminous matter with which they have been sensitized will be reported in a later paper.

We believe that the substance which sensitizes the animal is identical with that which later poisons it. However, the substance must first cause a reaction in the organism resulting in a production of antibodies. We have found that small quantities of horse serum produce, after a definite period of incubation, a condition of anaphylaxis; multiple or repeated injections produce immunity. We therefore possess in horse serum a substance capable of causing both anaphylaxis and prophylaxis.

It may be that man can not be sensitized in the same way that we have shown to be the case with guinea pigs. Children have, in a number of instances, been injected with antidiphtheric horse serum at short and long intervals without, so far as we are aware, causing death. Certain serums, for example, the antitubercle serum of Maragliano and the antirheumatic serum of Menzer, are habitually used by giving injections at intervals of days or weeks. In all such cases of frequent and repeated injections the amount which has been injected and the interval between the injections must be taken into account in relation to our work. Von Pirquet and Schick have shown that a second injection of horse serum into children causes an "immediate" or an "accelerated" reaction. Both the immediate and the accelerated reaction in children are characterized by symptoms of "the serum disease."

We might conclude that children may not be sensitized to the toxic action of horse serum by eating horse meat, for horse meat is a favorite article of diet in certain European countries and there is nothing upon record to show that the injection of horse serum in those countries is fraught with more danger than where this diet is not used. It should,

however, be borne in mind that our work has shown that guinea pigs may be sensitized with exceedingly minute quantities of a strange proteid, and that repeated injections cause an immunity, and it does not seem impossible that the same action may be true of food.

Man reacts to the first injection of horse serum after a period of eight to thirteen days; guinea pigs show no reaction as a result of the first injection; both man and guinea pigs react to a second injection. The reactions in man and the guinea pig differ, however, both in severity and in kind. The relation, therefore, that our observations upon the guinea pig may have in their application to man must await further study.

The fact that other animals beside man and the guinea pig react to a second injection of horse serum would seem to indicate that we are dealing with one and the same action.

We believe that our results make it probable that man may be rendered sensitive to the injection of a strange proteid, as is the case with the guinea pig and other animals, and that this explanation must be considered as well as the *status lymphaticus*, which has heretofore been assigned as the cause of sudden death following the injection of horse serum.

[Post Scriptum.—After the galley proof of this article had left our hands an article by R. Otto entitled "Das Theobald Smithsche Phänomen der Serum-Ueberempfindlichkeit," reprinted from Leutbold-Gedenkschrift, band 1, first came to our notice. His paper deals with some of the problems we have studied and his results are in harmony with many of our conclusions.]

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Immunology and NIAID (1887-1970)

Sheldon G. Cohen and William R. Duncan

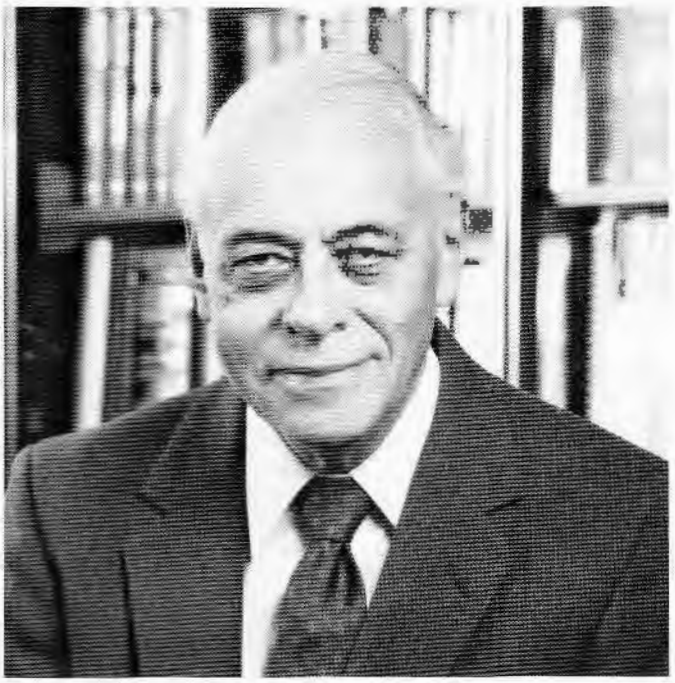
Rosenau, Anderson, and the Beginnings (1899-1915)

The first appearance of immunologic studies in the Hygienic Laboratory reflected the state-of-the art of the times. At the turn of the century, identification of the role of bacterial organisms in the definition of infection was just beginning to emerge as a new pursuit in the field of pathology. The unfolding of new knowledge offered corresponding new insights and opportunities to design specific attacks on unmasked microbial agents of disease, but not without creating a new array of problems and challenges for clinical medicine. In the evolution of this development, the revelation of immunologic phenomena in host defense was brought into focus within the Public Health and Marine Hospital Service.

It all began eighteen years after the Laboratory's establishment, during the tenure of Milton J. Rosenau, second Director of the Hygienic Laboratory. In 1899, after serving for twelve years as the Hygienic Laboratory's founding Director, Joseph Kinyoun left his position to join the H.K. Mulford Laboratories and Rosenau was named to replace him. Just eight years earlier, the Laboratory had been relocated from its original site in the Marine Service Hospital at Staten Island, New York, to a new facility in Washington, D.C. Here, as subsequent events would prove, the seeds were planted for immunology's growth and ascendancy to its current day position of preeminence among biomedical science endeavors housed within the National Institutes of Health—the ever-expansive, ultimate evolvement of that very same Hygienic Laboratory.

Rosenau, after receiving an M.D. degree from the University of Pennsylvania in 1889, pursued postgraduate studies at the Hygienische Institut in Berlin, the Institut Pasteur in Paris, and the Pathologisches Institut in Vienna. Commissioned in 1890 with the rank of Assistant Surgeon, he joined the Marine Hospital Service on return from training experiences at the famed medical centers of Europe.

Shortly after Rosenau had assumed the Directorship, an expansion of the Service was authorized in order to take on responsibilities in the newly-developing area of biological products that involved the preparation and standardization of vaccines and antitoxins.[1] This charge resulted from a tragic series of deaths in St. Louis attributed to tetanus contamination of administered diphtheria antitoxin. Working closely with Rosenau in this exciting initiative was John F. Anderson, who joined the Hygienic Laboratory as Assistant Director in 1902. Anderson, a medical school graduate of the University of Virginia, also had a European background of training in bacteriology at the medical centers of Vienna and Paris.



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Then, in 1898, following studies in England at the School of Tropical Medicine in Liverpool, he received a commission in the Marine Hospital Service.

In laboratory endeavors, deaths were being noted in guinea pigs used in protocols for standardization of diphtheria and tetanus antitoxins raised in foreign animal species. Relevant and especially frightening were reports of occasional severe reactions, and even some fatal accidents, that had occurred among patients treated with these antisera. Out of Anderson's undertaking to determine a possible common cause and basic mechanism for these incidents, classic studies on anaphylaxis were spawned in the Hygienic Laboratory. As a result, credit for the NIH's first landmark publication in immunology can be given to Rosenau and Anderson for their 1906 paper on the study of the cause of sudden death following injections of horse serum.[2]

The Laboratory's follow-up series of studies on the subject contributed measurably to the development of an understanding of anaphylactic phenomena.[3] It is pertinent to appreciate that these investigations were undertaken and interpretations established independently, without awareness of the dog and rabbit experiments of Richet and Portier in France in 1902. Additionally, Rosenau and Anderson characterized features of the mechanism of sensitization and demonstrated the requirements both for antigen-antibody specificity and for an incubation period of several days between sensitizing and shocking doses. They also noted the phenomenon of maternal transmission and the potential of the oral route for the induction of sensitization.

Although anaphylactic shock traditionally is referred to as the "Theobald Smith phenomenon" as a result of Otto's reference to Smith's unpublished observations, it is important to note that Rosenau and Anderson's findings likely represent the first valid demonstration of hyperreactivity in guinea pig experimental models. In contrast to the Hygienic Laboratory workers' critical experiments, factors of toxicity were evident in the induction of reactions in Smith's series, i.e. use of successively administered large volumes of horse serum and the absence of requisite sensitizing-challenging intervals. Smith's protocols were followed without control groups, and the observations lacked significance. Hence Rosenau and Anderson's contribution remains the first definitive publication on the subject of anaphylaxis. Also of interest is their introduction of the term "allergin."[3] However, it should be appreciated that this term, coined by the Hygienic Laboratory staff, referred to allergic antibody (also named "anaphylactin") and, thus, is not to be confused with "allergen," a current-day term used to define a sensitizing antigen in the etiology of atopic or classic human allergic disease.

Integrated with and a by-product of interest and involvement in the investigations of Rosenau and Anderson were the studies of William H. Schultz. After serving as an instructor in the Department of Physiology and Pharmacology at the University of Missouri, Schultz joined the Public Health and Marine Hospital Service in 1908 as Associate Pharmacologist at the Hygienic Laboratory. There Schultz's orientation to cardiac physiology and biological assay of drugs was directed to the study of the pharmacodynamics of anaphylactic shock. Out of this pioneering investigation came Schultz's classic contribution and landmark publication on contraction of the isolated strip of sensitized guinea pig ileum when suspended in a bath of physiological solution and challenged by specific corresponding antigen.[4] The following year, the English physiologist and pharmacologist Sir Henry H. Dale described a variation of this technique, utilizing the guinea pig uterine strip and its muscular contraction effected by β-iminazolyethylamine (histamine). Thereafter known as the Schultz-Dale phenomenon, the application of this technique found considerable use for many years in the study of local anaphylactic phenomena, identification of mechanisms and mediators of sensitization, and assay of pharmacologic anti-allergic and antihistaminic agents. After a stay of five years, Schultz left the Hygienic Laboratory in 1913 to accept the Professorship of Pharmacology and Materia Medica at West Virginia University.

When Rosenau accepted Harvard Medical School's invitation to assume the Chair of Preventive Medicine and Hygiene in 1909, Anderson was named the third Director of the Hygienic Laboratory. After publishing an exhaustive review on anaphylaxis,[3] Anderson turned his primary attention to other aspects of bacteriology, including cholera, typhus, tuberculosis, and poliomyelitis. Especially important was his collaboration with Joseph Goldberger, the Public Health and Marine Hospital Service officer who later achieved recognition as a pioneer in nutrition and the conquest of pellagra. Their work resulted in the development of an experimental animal model for measles by transmission of the virus to monkeys. Subsequently, in 1915, Anderson resigned from the Public Health Service to assume the directorship of E.R. Squibb and Sons Research and Biological Laboratories.

The In-between Years (1915-1957)

During the 32 years that followed Anderson's tenure, from immunology's standpoint, the Hygienic Laboratory and its successor, the National Institute of Health, continued to reflect the state-of-the-art of the times. In this evolving period investigations on immunity were undertaken with primary emphasis on unraveling the mechanisms of host- defense. Innovative studies on structure and function of the immune system would have to wait for the requisite base of information to be generated, first with the advent of applicable, sophisticated advances in biochemistry to provide the impetus for inquiry.

For all intents and purposes, the immunology of that period could be equated with its defined component of serology. The exquisite specificity of antisera was used as an aid in the study and classification of microorganisms and cleverly employed as a probe in unraveling the intricacies of pathogenicity. Highlighting the work of these

years were the contributions of Rolla E. Dyer[a], who in 1925 defined the unit for scarlet fever streptococcus antitoxin;[5] Kenneth F. Maxcy, who in 1928 published on infection and immunity in the epidemiology of diphtheria;[6] Ida A. Bengtson, who standardized gas gangrene antitoxin in 1934;[7] John Bozicevich, who in 1938 developed a reagent for the *in vitro* diagnosis of a helminth parasite infection;[8] and Karl Habel in 1940, who worked with yet another class of infectious agents, the rabies virus.[9]

Typical of the contributions from studies based upon serologic analysis was the definition of serotypes of various antigenic bacteria. As an outgrowth of these laboratory endeavors, investigators were provided with the means to identify microbial-derived specific antigenic components. In the case of the pneumococcus, the biochemically-isolated capsular polysaccharides provided opportunity for another landmark contribution from the NIH laboratories. For the first demonstration of "immunological paralysis" that subsequently evolved into the phenomenon known as immune tolerance, credit must be given the findings of Lloyd Felton in 1942.[10]

Upon receiving a P.H.S. commission in 1938, Felton joined the NIH Division of Infectious Diseases's staff after faculty positions in preventive medicine and hygiene at Harvard and in bacteriology and pathology at Johns Hopkins. Special interest in microbial infection, especially pneumonia, led him to undertake studies on the pneumococcus. From this line of investigation, Felton demonstrated the role of capsular polysaccharide in decreasing pneumococcal infection and the persistance of this microbial material in tissue, accounting for an expression of immunological paralysis, defined during his NIH tenure.

The demands of World War II years directed the attention of the NIH Division of Infectious Diseases professional staff to special needs of the military services, i.e. development of vaccines and the problems of diseases indigenous to tropical regions. Subsequently, with cessation of hostilities came the opportunity for recruitment of scientific staff from among the pool of those being discharged from military obligations. It then became possible for the expanding resources of NIH to support the new directions and expanding horizons of evolving biomedical disciplines. In this setting in 1948, a reorganized NIH gave birth to the National Microbiological Institute (NMI).

Although the NMI's mission in infectious diseases had the potential to expand beyond infection and microbial immunity, its horizons were still limited by the absence of a single focused unit capable of initiating investigative endeavors in a field that was beginning to take its own distinct shape as immunobiology and immunochemistry. Further, in 1955, a change in the NMI's name to the National Institnte of Allergy and Infectious Diseases (NIAID) added still another dimension to the Institute's mission. With the new component's aegis, NIH had come full circle in recognizing its roots in the pioneering endeavors of Rosenau and Anderson in hypersensitivity. The change from NMI to NIAID represented one in name, rather than in mission; for from its very beginning, implicit in NMI's charge were all aspects of infectious diseases, including invading organism and host response.

This nominal change from NMI to NIAID was in keeping with the pattern of identifying NIH component Institutes with disease categories. To the public, to concerned Congressional committees upon whose appreciation and insights NIH support depended, and to others of the laity working for health causes, microbiology as a descriptor evoked little in the way of practical understanding or sympathy for NMI's mission. The substitute insertion of infectious diseases would circumvent the lack of appreciation of a good number of illnesses that were inherent in the National Microbiological Institute's orientation. However desirable in designating the Institute's mission from a scientific standpoint, adding the descriptor *immunology* could be expected to accomplish little, if anything, in further clarifying the issue of the Institute's name to the laity. Instead, in the choice of *allergy* for the Institute's new name, consideration was given to the scientific acceptability of this term within a global interpretation that would include the study of immune functions and disorders. At the same time, it would satisfy those who believed that NIH had lacked involvement with and concern for that large segment of the public afflicted by allergic disorders.[b] Henceforth, the focus of the Institute's public visibility would be on an array of diseases within the new NIAID purview, rather than on a biomedical discipline, whatever the earlier intent to ascribe the very same role to the predecessor NMI. Nevertheless, it was some 18 years later that the first appearance of the singular name *allergy* became identified formally with an Institute laboratory section, and yet an additional six years later that a clinical section on allergy was created.[c]

It was in this setting that Dorland J. Davis[d] in 1956 assumed the position of Associate Director of NIAID and was given the responsibility for intramural research (a position later renamed Scientific Director). After graduation from medical school and advanced studies in public health at Johns Hopkins University, Davis received a commission as a PHS officer and came to NIH in 1939 to work in the Division of Infectious Diseases. Following World War II service as a medical officer in North Africa, he returned to NIH and in 1954 was appointed Chief of the Laboratory of Infectious Diseases. His background of experience in research on a variety of

[a] later served as NIH Director, 1942-1950

[b] Haas, V. H. Director, NMI, 1943-55; Director, NIAID, 1955-57, personal communication to the authors, 1987 (see note on last page of this chapter).

[c] the Section of Allergy and Hypersensitivity, initiated within the NIH Laboratory of Immunology in 1962, and the Section of Clinical Allergy and Hypersensitivity, established within the Laboratory of Clinical Investigation in 1968, are discussed in later sections of this account

[d] later served as Director of the National Institute of Allergy and Infectious Diseases, 1964-1973

parasitic and virus diseases provided him with additional insights to appreciate immunology's potential to assume an ever-expanding, interdisciplinary role in the basic biomedical and clinical sciences. It was, therefore, evident to Davis that the absence of focused immunologic research constituted a distinct void, and that a structured program could assume a prominent position in the facility that served as the nation's showcase of medical research endeavor.

At this time, moreover, the field of immunology had begun to expand with the emergence of new knowledge generated by research, but little in this area of investigation had developed within NIH. In later reminiscences, Davis noted[e] that, during the period of the late 1940s to the early 1950s at NIH, there were only two investigators dealing with immunologic areas, Lloyd Felton and Frederick G. Germuth. As previously noted, Felton's work on pneumococcal polysaccharides had provided experimental leads for his classic studies on immunological paralysis. In 1946, Germuth, in the position of Immunologist with a Laboratory of Infectious Diseases group housed at the Ft. Detrick facility in Frederick, had initiated a series of studies on hypersensitivity.[11] His interest in this area of research could be traced to earlier training and association in the Department of Pathology at Johns Hopkins with Arnold R. Rich, whose pioneering studies had centered on the delineation between immunity and hypersensitivity responses. Germuth subsequently left NIAID in 1951 for Washington University in St. Louis, the first of a series of positions in which his studies on immune complex phenomena and experimental vascular sensitization were extended. Although there was indeed a place for immunology, per se, there was an equal need for experts in immunologic research to interact, associate, and collaborate with counterpart basic and clinical scientists who were contributing to the widening spectrum of biomedicine at NIH.

During one of his periodic meetings with the Chiefs of the NIAID component intramural laboratories, Davis had occasion to introduce this subject. Following favorable discussion of Davis's proposal, a committee was charged with exploring all possibilities to secure a place for immunology in the Institute. Between two viable alternatives, the Committee favored establishing a new and independent laboratory of immunology, rather than promoting the generation of immunologic studies scattered throughout several existing laboratories. Establishing and implementing plans for a new unit and to direct studies at the fore of immunologic research, however, would require the proven leadership and experience of a recognized and accomplished immunologic scientist. Joseph E. Smadel, who had just moved from the position of Chief of the Department of Virus and Rickettsial Diseases at the Walter Reed Army Institute of Research to assume the Associate Directorship at NIH, was especially familiar with people in the field of immunology and in an informed position to offer the names of several candidates. On Smadel's recommendation, Jules Freund was recruited in 1957.

[e]personal communication to the authors, 1987.

Laboratory of Immunology: The Freund Years (1957-1960)

Jules Freund had had a long and distinguished career as a medical scientist that began on the faculty of preventive medicine at the University of Budapest. He then served as bacteriologist at the Von Ruck Research Laboratory and the Henry Phipps Institute of the University of Pennsylvania, as a member of the faculty of Cornell University Medical College Department of Pathology, and as Assistant Director of the Bureau of Laboratories and Chief of the Division of Applied Immunology at the Public Health Research Institute of the City of New York. He was well known at NIH in his role as a consultant to the Public Health Service, as a past president of the highly selective American Association of Immunologists, and for his original studies on antibody formation, toxin-antitoxin reactions, and autoimmunity in aspermatogenesis. He was also credited with the development of the most potent agent known to the present day for the enhancement of immune responsiveness.[f,12]

These were the early years of the unraveling of knowledge of biochemistry applicable to the new dimension that quantitative methods were bringing to chemical immunology. With an appreciation of immunochemistry and yet not neglecting beginning revelations in cell-mediated immunity, Freund justified the confidence placed in his appointment. As remembered by Davis and former associates, Freund proved to be the right person to assume the requisite responsibility for the development and direction of the Laboratory of Immunology. The respect for his fund of knowledge was enhanced by the personal element of character he brought to the position. Although scientifically demanding in maintaining standards of exactness and excellence, at the same time Freund was ever ready to extend assistance to associates and colleagues with gentility. Against the background of firm, thoughtful leadership by a keen, expertly informed scientist and kindly man, NIAID's Laboratory of Immunology was formally initiated in a setting of harmony, excitement, and mutually-supportive efforts.

One of the very important and evident strengths that Freund brought to the construction of the Laboratory of Immunology (LI) was his ability both to identify and to bring together those with expertise whom he found in place within NIH and to guide those he had recruited in undertaking original pursuits with immunologic bent. Constituting the original LI staff were the one associate who had accompanied Freund from New York, Sanford H. Stone, and three previously established NIH medical scientists, John Bozicevich, Sheldon Dray, and John E. Tobie. Although these appointments, effected through a regrouping process, represented a spectrum of varying experiences and orientations, a common denominator of interest and purpose guided the shaping of the Laboratory's thrust. At the outset, a deliberate decision

[f]Freund's adjuvant, consisting of killed mycobacteria, an emulsifying agent, and mineral oil for the incorporation of an immunizing antigen.

was made by Freund and those administratively concerned to focus on and emphasize laboratory research in basic immunology. The fact that this plan for the Institute's innovative venture was designed also to provide the foundation for an ultimate clinical arm was evident in the following notation that appeared in the NIAID Annual Report of Program Activities for 1957:

> The new research program of allergy and immunology is one year old. The immediate objectives have been to initiate research projects in problems which could be attacked by laboratory methods. These are now underway and the new Laboratory of Immunology looks towards the beginning of clinical studies concurrently conducted with fundamental laboratory investigations.

With this mission in view, the recruitment process for additional staff with appropriately relevant interests began. Among the first new names to be added in 1958 were those representing a younger group invited to embark on the new NIAID venture: Wilton E. Vannier from the California Institute of Technology, Curtis A. Williams from the Rockefeller Institute for Medical Research, Philip Y. Paterson from the University of Virginia, and Philip R.B. McMaster, just out of medical training at the Cornell-New York Hospital Medical Center.

Upon setting themselves up in NIAID's new Laboratory of Immunology, Freund and Stone continued the line of investigation they earlier had pursued in New York. This had consisted of studies on animal models of adjuvant-induced immune-mediated disease, including aspermatogenesis as the first fully authenticated pathologic demonstration of experimental autoimmunity, experimental allergic encephalomyelitis, and thyroiditis. In 1958 the more classic concept of allergy first appeared at NIAID with Stone's investigations on experimental anaphylactic shock, representing an extension of earlier work initiated with Freund.[13]

It was during this period that increasing attention was being directed to the guinea pig as an especially suitable experimental model for the study of delayed-type hypersensitivity. Adoptive cell transfer of this immune-mediated phenomenon by lymphoid cells from sensitized donors to naive recipients had recently been demonstrated in the Rockefeller Institute laboratories of Merrill W. Chase. Freund, perceiving that genetic control could influence the outcome of such experiments, uncovered a unique opportunity in a NIH resource that for some time apparently had been forgotten. Dating back to the work of the geneticist G.M. Rommel in 1906 at the Department of Agriculture, perpetuation of the two surviving lines of inbred guinea pig strains, numbers 2 and 13, had been continued as a matter of routine by a NIH animal technician. When he learned of the existence of this non-utilized animal colony, Freund appreciated the great advantage inherent in homozygous states for studying lymphoid cell functions; accordingly he seized the opportunity to develop applicable protocols. The resultant series of Freund and Stone experiments, based on the efficacy of cell transfer of tuberculin sensitivity and allergic encephalomyelitis, demonstrated the special value of the inbred guinea pig in the study of cell-mediated immunity. In later years, these inbred strains resurfaced and, when brought to the fore of immunologic research by Freund and Stone, proved especially valuable to NIH scientists in pioneering studies on genetic control of the immune response.

Of the original Laboratory of Immunology Staff, Sheldon Dray had first served as a P.H.S. commissioned officer for two years in the Nutrition Section of the Bureau of State Services. Next joining the Physical Biology Laboratory of the National Institute of Arthritis and Metabolic Diseases in 1949, Dray initially focused studies on the physical chemistry of membranes and membrane transport. Newly-developing interests in non-infectious immunology then led him in 1955 to seek transfer within NIH to the NIAID Laboratory of Clinical Investigation. In this setting, Dray planned to take up his earlier interests in antigen-antibody reactions, and in the field of allergy to give special attention to chemical procedures for the isolation of ragweed pollen antigenic fractions. These were subject areas to which he had been introduced in the laboratory during medical and graduate school years.[g]

In another redirection of orientation, Dray's interest again was diverted from attraction to the ragweed system to the potential of the technique of immunodiffusion that recently had been developed by Jacques Oudin.[h] First to gain experience at NIH in this new technology and its utilization in the separation and study of serum proteins, Dray soon served as a NIH-wide resource for the work of colleague scientists. By coincidence, this occurred about the time Oudin came to NIH as a visiting foreign scientist. Mutual interests in this common scientific endeavor led to additionally rewarding interactions and Dray was influenced to pursue yet another line of investigation that Oudin had been pioneering, allotypy. Then an invitation to join Freund's Laboratory of Immunology facilitated his undertaking these new studies.

As in Freund's case, Dray found in NIAID a unique animal research resource, a large breeding colony of rabbits that provided the necessary stock for protocols designed to study allotypy. Unfortunately, reality offered the first roadblock. Dray learned that the large numbers of animals required for this line of investigation were housed in a facility intended only for production and supply, not for research. However, with administrative insight, this issue was resolved. In this unique setting, Dray not only confirmed Oudin's seminal findings but also expanded the scope of this particular area of immunogenetics. In attempting to extend these studies to the definition of human allotypes, Dray employed protocols based upon immunization of primates with human sera, and, as a fortuitous by-product, discovered IgG subclasses.[14]

[g] As a graduate student with William H. Welker, Chairman of the Department of Physiological Chemistry at the University of Illinois College of Medicine and a pioneer in the field of immunochemistry.

[h] Chief of the Analytic Immunochemistry Service of the Institut Pasteur, Paris, 1959-1978.

One important contribution to medicine that was especially concerned with clinical laboratory endeavor originated in the Laboratory of Immunology through the work of John Bozicevich. His description of the technical method for adsorbing DNA to bentonite particles represented an innovative approach to serologic identification by visible flocculation. This procedure, which lent itself to the detection of anti-DNA antibodies, was found to have extremely useful applications as a laboratory aid in the diagnosis of systemic lupus erythematosus and rheumatoid arthritis.[15] Reported in Bozicevich's article—which evoked requests for 3,000 reprints—the technique was widely used in hospital and clinical laboratories throughout the country. A co-author in this publication, Donald E. Kayhoe, at the time a LCI senior investigator, subsequently joined the NIAID Collaborative Research Program in 1966 as head of the Transplantation Immunology Branch (TIB). Through the work of the TIB, NIAID pioneered the development of a histocompatibility typing serum bank, as a unique extramural research resource for the scientific community, and the large-scale, multicenter Kidney Transplantation Histocompatibility Study.

Coming from an appointment in tropical medicine at Tulane University in 1943, John Tobie's first association with NIH in the Division of Zoology lasted but a few months. Entering military service that same year, Tobie joined the U.S. Army Sanitary Corps and was assigned to the South Pacific theater as Chief of the Department of Parasitology in an Army medical laboratory. Upon returning to NIH, he worked in the Division of Infectious Diseases' Laboratory of Tropical Diseases (later named the NIAID Laboratory of Parasitic Diseases) on serologic flocculation and complement fixation studies in parasitic diseases. After joining Freund's group, Tobie became more involved with basic immunology and in this area was responsible for introducing the technique of fluorescein attachment to antibodies.[16]

The newly-recruited group of Vannier, Williams, and Paterson were placed in a curious circumstance: the facilities of the Laboratory of Immunology were not ready to provide adequate laboratory space for all. An interim plan was developed. Although carried on NIAID staff rolls, by special arrangements they were temporarily detailed to extramural laboratories and given concurrent visiting or affiliated institutional appointments. Through specifically-effected contracts to the cooperating institutions, NIAID paid their official salaries and provided support for the research projects undertaken.

Prior to his NIAID recruitment, Vannier had pursued advanced training at the California Institute of Technology with Dan Campbell, a pioneer in immunochemistry and in the development of the basic science foundations of allergy. Under the terms of the temporary arrangement, Vannier's LI appointment was initiated while he continued to work in Campbell's laboratory on the isolation and characterization of allergenic fractions of house dust.

Paterson had come to Freund's attention through Paterson's earlier associations with Smadel at the Walter Reed Army Institute of Research and with Lewis Thomas at Tulane University. The Thomas-Paterson studies on experimental allergic encephalomyelitis (EAE) served as a link with Freund's identical research interest in autoimmunity. Until adequate animal quarters for Paterson's proposed investigation could be put in place at NIH, Freund arranged for him to work with Alwin M. Pappenheimer, Chairman of the Department of Microbiology at New York University (NYU), on delayed-type hypersensitivity. While at NYU, Paterson had demonstrated to Freund that EAE could be induced without mycobacteria in the mineral oil adjuvant (Freund's incomplete adjuvant) if xenogeneic, rather than allogenic, spinal cord was used as the autoimmune inducing antigen; also, Paterson was the first to adoptively transfer EAE.[17]

In New York, Nobel Laureate Rene J. Dubos and the Rockefeller Institute provided the Freund-Williams connection. Freund had known Williams and was familiar with his studies on mycobacteria with Dubos and his earlier work on immunoelectrophoresis. Williams's LI appointment by interim assignment in Dubos's Rockefeller laboratory and his pursuit of studies on immunologic properties of methanol extracts of tubercle bacilli constituted another link with Freund's research interests.

McMaster, in his neophyte scientist status, began work at NIAID on experimental models of autoimmunity to complement the thrust of Freund and Stone's ongoing studies. Of the off-site group, Vannier subsequently did come to NIH in 1960 to continue and further his work in immunochemistry. Paterson and Williams, however, terminated their NIAID appointments after two years.

It was anticipated that construction of the requisite space and facilities at NIH would be completed within a year after Paterson's arrival at NYU in 1957, but by 1959 this still had not been effected. Paterson's decision to resign his LI position was further influenced by Freund's development of serious illness and the offer of a faculty appointment by his former Tulane mentor Thomas, who had since come to NYU and taken over the chairmanship of the Department of Medicine. When Freund died in 1960, it became apparent to Williams that a new Chief of LI would not be appointed for some time. Preferring a more clearly defined option, he resigned from his NIAID position in favor of remaining for an appointment at Rockefeller with Edward Tatum in the Laboratory of Biochemical Genetics. Thus, the Paterson and William episode constitutes a unique experience in the history of NIAID immunology. Although their publications appeared under the official NIAID aegis, except for periodic visits to prepare quarterly reports and discuss progress with Freund, they enter the record as active scientific staff who had never worked within the structural confines of the Institute.

As programs in basic immunology were taking shape in the Laboratory of Immunology, Freund began to look towards possibilities for developing clinically-relevant investigations. In this connection, his attention was drawn to Howard C. Goodman, who was moving into immunology from a clinically-oriented base. Goodman's initial NIH appointment in 1953 was as a research associate at the National Heart Institute (NHI), where he

came after serving as a research associate at the Cedars of Lebanon Hospital Institute of Medical Research in Los Angeles. At NHI, with James A. Baxter, Chief of the Laboratory of Cellular Physiology and Metabolism, Goodman's work involved studies on experimental Masugi nephritis. Additionally, his studies identifying serum factors in patients with lupus erythematosus brought him into collaboration with immunologists within other NIH component Institutes.[i] Especially important were associations with John L. Fahey of the National Cancer Institute (NCI)[j] and Sheldon Dray of NIAID. Influenced by these interactions, Goodman recognized that his interests were turning increasingly to immunology and appreciated the fact that further training would be required if he were to progress in this area. Consequently, he arranged to spend a year (1959-1960) in the laboratory of Pierre Grabar at the Institut Pasteur.

While in Paris studying serum proteins, utilizing Grabar's pioneering technique of immunoelectrophoresis, Goodman received an invitation from Freund to join the NIAID Laboratory of Immunology on his return to NIH. He eagerly accepted this opportunity, but a short time later Freund developed a terminal illness and died before Goodman completed his stay with Grabar. Nevertheless, upon return to NIH in 1960, Goodman followed through on original plans and joined the Laboratory of Immunology whose direction had been since given to John Tobie as Acting Chief.

In retrospect, the three years of Freund's tenure are remembered and recognized as the period when immunology gained its own identity within NIAID. In addition to bringing the LI into organizational being, Freund left his personal mark indelibly stamped on the shape and direction taken by immunology as it developed into an independent Institute endeavor, beyond and apart from microbial infection. Insightful guidance and encouragement of initiative characterized Freund's quiet, but strong, influence on associates and investigative undertakings. At the same time, his otherwise optimistic plans for the start-up process were tempered as Freund faced the realities of limitations on available space and positions. The placement of newly-joined junior staff who attempted to initiate their work and expand their horizons at distant laboratory sites that were separated from day-to-day contact and vital interactions with NIAID mentor and colleagues, and, ultimately, the appearance of terminal illness reduced Freund's active role as LI Chief to even less than his three years of record. Were it not for these circumstances, perhaps a more lengthy documentation of productivity and objective accomplishments in the building process, and a more successful outcome in the recruitment and retention of new staff might have been possible. Regardless, the contribution of the Freund years is unquestioned: a new disciplinary effort at NIAID was launched and the pattern of its laboratory base set to keep pace with the advancing forefront of immunobiology and immunochemistry.

Laboratory of Immunology: Tobie and a Holding Action (1960-1962)

All concerned at the leadership level had agreed that the course set by Freund for the Laboratory of Immunology should be continued. The search for a Laboratory Chief would concentrate on identifying and recruiting a scientist with an extensive background of experience and credentials in basic immunology. For the interim, Tobie agreed to take on the responsibilities of Acting Chief. For the next two years, this arrangement worked well and to the satisfaction of all involved in contributing to the Laboratory's best interests. During the transition period, the work of the LI progressed. Stone's studies on anaphylactic shock and experimental allergic encephalomyelitis were furthered, and Dray's extended studies on allotypy resulted in the description of allotypic suppression and the advancement of a new immunologic concept.[18]

An important and innovative development during the two-year Tobie period was the staff initiative that effected meaningful, interactive efforts between the Laboratory of Immunology and the Laboratory of Clinical Investigation (LCI), then under Vernon Knight as LCI Chief. Not only were collaborations effected, but also organizational lines were crossed and official joint appointments were made. Although maintaining his primary staff assignment in the Laboratory of Immunology, Goodman's concurrent association with the Laboratory of Clinical Investigation was made formal when a position was created especially for him. Identified as Head of a newly-established Section on Clinical Immunology, he played a pivotal role in establishing this section as a new prototype and model for clinical immunology units in research and academic institutions. Before leaving NIAID in 1963 on detail in Geneva to the World Health Organization as Chief of its Immunology Unit, Goodman focused his studies on experimental thyroiditis, purification of human serum gamma globulin, and the interaction of serum factors from lupus patients with chromosomal material.[19] The first demonstration of antibody activity of certain immunoglobulin classes and subclasses by Fahey and Goodman[20] was representative of NIH's developing opportunities and unique resources for collaborative efforts. It was just such endeavors, crossing organizational lines of the categorical Institutes, that measurably contributed to immunology's disciplinary expansion and increasing relevance to clinical medicine.

A new recruit joining the Laboratory of Immunology during this period was Arthur J.L. Strauss, who, although a neophyte investigator, already had an established relationship of his name to a seminal observation. Having developed a special interest in neurological diseases during a postdoctoral fellowship at Columbia

[i] research in basic immunology at the National Cancer Institute was especially concerned with immunoglobulins, whereas studies at NIAID tended to focus progressively on areas of cellular immunology.

[j] After leaving the NCI in 1971 to take up the position of Chairman, Department of Microbiology and Immunology at the University of California, Los Angeles, Fahey served NIAID as a consultant in several areas relevant to the Institute's mission in Allergy and Immunology, including membership on the National Advisory Allergy and Infectious Diseases Council.

University, Strauss, with co-workers, undertook clinical investigative studies on myasthenia gravis. In 1960 he was the senior author of a publication reporting the original demonstration of a muscle-binding complement-fixing globulin by immunofluorescence. Eager to pursue and extend this productive line of investigation, Strauss arrived at the Laboratory of Immunology in 1961, specifically to work with Goodman. During the ensuing years, Strauss's studies were primarily focused on myasthenia gravis as an immunologic disorder, and by 1962 he could report on serologic studies from 100 myasthenic patients.[21]

Tobie's own work also was furthered by collaborative efforts with the Laboratory of Clinical Investigation. In 1960, Sanford Kuvin arrived at NIAID to begin an appointment as Clinical Associate in the Laboratory of Clinical Investigation. During a previous period of graduate school studies in medical microbiology at the University of Pennsylvania, he had developed a special interest in immunology. However, at NIAID, he found that immunologic investigation relevant to his area of investigative interest in parasitic infection had yet to be undertaken. At that time, studies in the Laboratory of Parasitic Diseases centered on the morphology of organisms and those in LCI on other clinical features of parasitic diseases. By initiating a collaborative association with Tobie and applying the latter's technologic approaches, Kuvin launched a series of immunologic studies on antibody responses in human malarial infection. Special credit was given to this LI- LCI group for demonstration of the first reliable technique for the detection and titration of human antibody responses to malarial organisms by the indirect method of immunofluorescence.[22] In 1963, Kuvin left for the London School of Tropical Medicine and Hygiene to further special scientific and professional interests that had been nurtured at NIAID.

Laboratory of Immunology: The Landy Years (1962-1967)

To aid in the recruitment of a permanent Chief for the Laboratory of Immunology, a search committee was formed. Several able external candidates were identified and contacted. Although some candidates expressed initial interest in exploring possibilities, serious negotiations were another matter. In addition to the ever-present deterrent of salary reduction that senior academic scientists had to face when electing to enter government service, another factor contributed to the attractiveness of external candidates' existing situations. These were the bright, beginning years of support for immunologic research. Because the outlook for continued availability of ample, if not generous, NIH grant awards for extramural research was optimistic, potential candidates were provided with a valid reason to decline consideration. Attention then turned internally to one of the search committee members, Maurice Landy, who, at that time, was Head of a National Cancer Institute Section on Immunology. Ultimately, Davis offered the position to Landy, and in 1962 a new Chief for the Laboratory of Immunology was in place.

Landy had come to NIH six years earlier from the Walter Reed Army Institute for Research, where he served as Chief of the Department of Bacterial Immunology. As head of the Section of Immunology of the National Cancer Institute's Laboratory of Chemical Pharmacology, his research interests were focused on mechanisms of host defense, especially on the role of microbial products in precocious and natural immunity.

When Landy assumed directive responsibility, the Laboratory's primary thrust began to reflect his interests in natural immunity.[23,24] However, other areas of basic immunology that were in the process of accelerating ascendancy to the forefront of the biomedical sciences were not neglected. Sections were created to accommodate and further the special interests of senior staff.

Tobie, who had served in an acting capacity during the previous two years assumed responsibility for a newly-created Section on Applied Immunology within the LI. The following year, in further pursuit of special interests, Tobie moved to the NIAID Laboratory of Germfree Animal Research as Chief and served in this capacity until retirement in 1972. Additionally, in 1969 he took on concurrent responsibilities as NIAID Assistant Scientific Director for Laboratory and Clinical Research.

In addition to the Section on Applied Immunology for Tobie's work, research on autoimmunity previously introduced by Landy's predecessor, Freund, was continued by Stone in a Section on Allergy and Hypersensitivity. Others Sections included Immunochemistry, headed by Dray; Clinical Immunology, headed by Goodman in association with the LCI; and Autoimmunity with Strauss.

In the Section on Immunochemistry, Dray continued studies on immunoglobulin allotypes. His report on allotype suppression stands as an especially important contribution to this subject.[25] The influence of Dray's development of this research area at NIAID was felt as intramural laboratory lines were crossed in collaborative efforts with LCI staff.[26] In 1965, Dray left NIAID to head the Department of Microbiology at his alma mater, the University of Illinois College of Medicine, and was succeeded by Vannier as Head of the Section on Immunochemistry.

The line of investigative work that Dray had initiated was carried forward by one of his former trainees, Rose Mage. Mage had come to NIH as a postdoctoral fellow in the Laboratory of Immunology after completing work for a Ph.D. with Elvin Kabat at Columbia University.[k] In view of her primary interest in genetics, Mage was attracted to special studies on allotypy under Dray's direction and, in 1964, she received a staff appointment as Career Investigator. In this area of endeavor, her studies included allotype suppression and the genetic and chemical basis of antibody diversity. Especially noteworthy was Mage's contribution to understanding the organization of genetic information coding for the variable and constant regions of immunoglobulin chains.[27]

[k]Professor of Microbiology, Human Genetics and Development at Columbia University College of Physicians and Surgeons. Kabat's formal affiliation with NIH was later initiated as a Fogarty Institute Scholar in 1974, later with a NCI appointment as Expert in 1975, and continued since 1981 at NIAID. In this connection, his studies have been concerned with the development of a computer base containing published amino acid and nucleotide sequences of immunoglobulins and other immunologically relevant molecules.

A major Landy recruit joining Dray in the Section on Immunochemistry in 1965 was Ralph A. Reisfeld. After receiving his Ph.D. in 1957, Reisfeld spent two years at NIH in the position of Biochemist in the NCI Endocrinology Branch. Four years as a chemist in the pharmaceutical industry with Merck and Company followed, after which he joined Dray in the LI Immunochemistry Branch. An analytic chemist, Reisfeld collaborated with Dray on chemical characterization of rabbit immunoglobulin allotypes and their correlation with biologic function of genetic determinants. He also worked with Mage on chemical differences among the different allotypes. After Dray's departure, Reisfeld undertook independent studies on the isolation and characterization of transplantation antigens and contributed some of the first biochemical information on human histocompatibility antigens.[28] Additionally, his LI investigations on structure and function of human tumor-associated antigens, centering on malignant melanoma, provided the foundation for future years of work at the Scripps Clinic and Research Foundation after leaving NIAID in 1970.

Another Landy recruit who made major contributions to LI research endeavors in immunochemistry was John Inman. Following work in the pharmaceutical industry with Ortho and with Johnson and Johnson, Inman held a special NIH fellowship at John Hopkins, after which he joined NIAID in the position of Research Biochemist. During this early period in the LI, his work and contributions were concerned largely with the development of innovative technologies, e.g. immunoabsorbants and methods for peptide isolation, sequencing and synthesis.[29]

Within the Section on Autoimmunity, Strauss advanced his inquiries on the immunologic aspects of myasthenia gravis, especially anti-muscle factor (AMF), with whose identification he had been credited. Out of this work came the first indication of the reactivity of AMF with identical components found in thymic myoid cells and skeletal muscle.[30] This finding was of considerable clinical interest in view of the association of thymomas and frequency of thymic hyperplasia in patients with myasthenia gravis.

An early problem Landy faced was in recruiting additional staff. He sought to expand the LI's research horizons by creating a "critical mass" of investigators with appropriate diversity and experience. The aim of this infusion of additional talent was to enhance productive interactions among staff. However, it was difficult to attract qualified, established investigators with the requisite expertise and sophistication. First, there were limitations on salary and level of career positions built into the Federal government system that made it extremely difficult, not only to attract, but also to retain accomplished senior medical scientists. Second, a ceiling on allotted positions further complicated this issue. Although the LI had been provided with a more than adequate budget, most of it was designated for supplies.

In this regard, however, Landy proved to be as resourceful and inventive as he was imaginative and insightful. When competitive salaries and positions could not be offered, he brought in visiting foreign scientists and guest workers supported by external funds from pharmaceutical companies and international organizations, e.g. Rotary International. In this undertaking, Landy's wide-ranging international associations were revealed. Among the collaborations between notable visiting scientists and LI staff were those of Ruggero Ceppellini of Italy with Landy on bacterial polysaccharides,[31] Payotis Liacopoulas of France with Stone on anaphylaxis,[32] Jan B. Borjeson of Sweden with Reisfeld on pokeweed mitogen,[33] and Gideon Goldstein of Australia with Strauss on the thymus in myasthenia gravis.[34] Also included was Shlomo Ben-Efraim, who extended the series of studies initiated in Israel on synthetic antigens,[35] and from Czechoslovakia, Tomas Hraba and Jaroslav Rejnek.[36] Not only did a qualitative change occur, transforming the intellectual environment by providing opportunities for collaborations and interchanges in this international-like atmosphere; also a quantitative factor soon became apparent. By 1965, the European visiting scientists outnumbered the LI permanent staff until the addition of more career positions restored the balance.

The LI Clinical Immunology Section, as originally conceived by Freund with Knight's support, with provision for active associations with the Laboratory of Clinical Investigation continued to be productive into the early Landy years. In addition to Goodman's own work, there were evident spin-off benefits. Clinical Associates in the LCI who wished to gain experience in immunologic research had opportunities to work with Goodman on immunosuppression, with Landy on endotoxin, with Dray and Vannier in immunochemistry, and with Stone on autoimmunity and hypersensitivity.

When Goodman left the LI to take up his WHO appointment in Geneva, Landy sought to replace him in the position of Section Head. However, as an experimental laboratory with a non-physician chief, the LI could not be provided with beds in the NIH Clinical Center. Without the promise of access to patient involvement for the hands-on study of disease entities, Landy was unsuccessful in recruiting attempts to fill the void created by Goodman's departure, and the original plan gradually dissipated. Elsewhere within the LCI, the study of immunologic diseases generated increasing staff interest, and a structured program on clinical immunology and allergy eventually emerged. An account of this development is given in a following section.

In retrospect, it was only to be expected that a change in LI leadership would be accompanied by a variety of organizational and functional changes reflecting the new Laboratory Chief's scientific interests and goals and his personal and operational style. Where Freund of necessity had to focus on initiating the process of organization and Tobie on maintaining progress, Landy was presented with the opportunity to further and expand the Laboratory's development and to influence the direction of its investigative components.

In the LI that Landy took over, he perceived the existence of only a beginning. He appreciated the need to consolidate and strengthen existing resources, introduce new initiatives and fill the voids left by the incomplete

realization of Freund's plans for staff development. The creation of LI Sections and designation of established investigators to continue independent pursuits of special interests as Section Heads kept the LI on course. At the same time, this move minimized any sense of threat and overshadowing by the new Laboratory Chief's introduction of studies on microbial-related immune phenomena as an area of major research emphasis. Yet it would have been quite unrealistic to expect that a change-over to new style, objectives, and design could have been made without creating unrest and a whole new set of stresses.

The accelerating pace of research advances in immunology required those who would move with the field to generate new insights and exploit newly-developed experimental leads. In retrospect, a forward-looking, expertly-informed Laboratory Chief with evident convictions hardly could be less demanding of the potential given to his charge than offered by the opportunities and challenges of the times. Quiescence had passed with the Freund years; Landy and staff shared in the ferment of intellectual forces, expressions, and differences that characterized the Laboratory of Immunology in transition.

As Landy approached his fifth year in the position of LI Chief, a shifting of scientific and career interests was in the making. He had worked to move the LI from the fading era of descriptive immune phenomenology to a more demonstrably analytic and theoretical discipline. Further, the ever-expanding field of immunology was rapidly providing new knowledge on the biology, chemistry, and genetics of the system of cells responsible for immune function. Landy's perceptions and insights led him to appreciate that within this changing biomedical science lay opportunities for intellectual excitement and reward beyond the actual conduct of laboratory-based original investigation. To him, NIAID appeared to offer just the right setting for judicious utilization of unique resources and for involvements to influence and enhance the development of the field.

With John R. Seal,[1] who had since replaced Davis in the position of NIAID Scientific Director, Landy began to explore possibilities. Both Landy and Seal agreed that to meet the challenges of immunology's emerging advances, NIAID needed an informed and accomplished scientist to direct the Institute's extramural research program in this field. Accordingly, in 1967, Landy undertook the task that required a career change and moved into the position of Chief of the Allergy and Immunology Branch of the NIAID Extramural Program. In this capacity, until his retirement from NIH in 1972, Landy utilized this sophisticated base and his wealth of associations to excellent advantage. Especially noteworthy were his unique contributions and assistance to biomedical science through his role in designing the extramural network on Program Projects on Lymphocyte Biology and Brook Lodge conference series. The latter highlighted new, advancing research areas of the day, i.e. mediators of cellular immunity, immunologic intervention, immunological tolerance, immune surveillance, and genetic control of the immune response.

Laboratory of Immunology: The Benacerraf Years (1968-70)

With Landy's move to take on new responsibilities and challenges of the NIAID Extramural Allergy and Immunology Branch, the position of Laboratory of Immunology Chief was once again open. John R. Seal, as Intramural Scientific Director, had definite convictions as he simultaneously viewed both the rapidly-changing field of immunology and the considerable intellectual resources of the NIAID. Within the LI he perceived the potential to move to the forefront of advancing research in the field it represented to the community of basic biomedical scientists. For the LI in transition, the search was on for strong, imaginative leadership combined with scientific expertise. But first, the direction most advantageous for the LI to take needed to be charted.

After surveying the rapidly-changing field of immunobiology, in the Search Committee's judgment cellular immunology and immunogenetics were pinpointed as the particular areas offering the greatest potential and opportunities. To meet this objective, Baruj Benacerraf of New York University was identified and the recruitment process was initiated.

In his presentation to Benacerraf, Seal emphasized NIH's character as an institution dedicated solely to research and research training, the availability of resources that at the time seemed almost limitless, and the benefits associated with scientific interactions in a large, multidisciplinary, intramural program. An additional attraction was the availability of the NIH large colony of inbred guinea pig strains that earlier had caught Freund's attention and that was important for Benacerraf's pioneering studies on immune response genes.

As in the case of the LI's founding Director, Freund, Benacerraf came to NIAID already established as a major figure in the research world of immunology. For the previous twelve years he had been a member of the nationally-recognized group of immunologists assembled at NYU by Lewis Thomas. During that expansive, exciting period, Benacerraf had established a strong research program on genetic control of the immune response and cellular interactions involved in the induction of antibody production. These would be the areas he would continue to pursue during his forthcoming tenure at NIAID.

With the thrust of LI research turning in the direction of cellular immunology and immunogenetics, a realignment in Institute programs ensued. One change of interest was the relocation of investigations on autoimmunity, one of the earliest studies in LI's history. Stone in 1969 transferred the setting for his studies to a newly-created Section on Immunology in the Laboratory of Microbiology. In 1968, following newly-developing interests, Strauss pursued training in psychiatry and joined

[1]When Dorland Davis was named NIAID Director in 1961, John Seal (Capt. M.C., U.S.N.), then Director of the Naval Medical Research Institute in Bethesda, was recruited for the position of Deputy Director for Intramural Research (title later changed to Scientific Director in 1969). Seal later served as Acting Director of NIAID in 1975, following Davis's retirement, and as Deputy Director (1975-81) during the subsequent tenure of Richard M. Krause.

the staff of a PHS neuropsychiatric facility, St. Elizabeth's Hospital in Washington, D.C.

During the early Benacerraf period, research studies focused on several areas of basic immunology, primarily the genetics and chemistry of immunoglobulins, genetic control of immune responses, and characterization of fundamental mechanisms of cellular immunology. Continuing in LI were the investigations of Mage, Reisfeld, and Inman. There was Mage's demonstration of separate loci that encode for the variable and constant regions of rabbit IgG heavy chains.[27] Reisfeld, with expertise in analytic immunochemistry and in collaboration with Mage, pursued studies on the characterization of rabbit immunoglobulin chains at immunochemical and biochemical levels and the definition of chemical differences between allotypes.[36] Other areas of Reisfeld's work, concerned with the isolation and characterization of transplantation antigens, contributed some of the earliest information on the biochemistry of human histocompatibility antigens.[28]

In Rose Lieberman's ongoing studies, initiated in the LCI, Benacerraf identified an area relevant to LI interests to be encouraged and furthered. Accordingly, Lieberman was recruited to the LI; with her transfer, the studies of Mage and Lieberman provided the LI with a strong program on the genetics and biochemistry of immunoglobulins. In Lieberman's collaborative series with Potter of NCI, important contributions were made in studies on murine immunoglobulin heavy chains.[m,37]

In line with the aims of the LI reorganization, Benacerraf recruited two of his former NYU trainees, who were in the process of developing independent careers as investigators, William E. Paul and Ira Green. For Paul, this constituted a reassociation with NIH, since he had served as a clinical associate in the NCI Endocrinology Branch in 1962-64. Green's credentials in basic immunology were supplemented by a clinically-relevant research background in hematology.

Of Paul's research interests in cellular immunology, he focused on cellular interactions in the immune response and to antigen-binding receptors of leukocytes. With Benacerraf, Paul described the role of carrier-specific cells in anti-hapten responses.[38]

While at NYU, Green had initiated studies on genetic control of the immune response. Continuing this line of investigation with Benacerraf in the LI, he demonstrated the limiting influences of the major histocompatibility complex on immune response (Ir) genes in guinea pig models.[39] In another rewarding collaboration, Green and Frank of LCI opened up a new area for extended study in their demonstration of the C4 deficient guinea pig strain.[40]

Benacerraf's tenure as LI Chief covered only a two-year span. During this period, he continued to extend the lines of investigation initiated earlier at NYU. Especially inventive was the series of studies based upon his earlier seminal observation on Ir genes that probed the genetic control of the immune response to simple antigens. With Green and other LI associates, he documented the close linkage of Ir genes and the guinea pig major histocompatibility complex (MHC).[39] This line of investigation, extended into later post-NIH years, earned Benacerraf the Nobel Award for Physiology or Medicine in 1981. Additionally, during his LI years, he initiated another pioneering, experimental series on the cellular collaboration required for induction of immune responses to hapten-carrier complexes.[41]

Having fulfilled his commitment to reorganize and further the development of a vibrant and highly productive Laboratory of Immunology, Benacerraf left NIH in 1970 to accept the Chair in Pathology at Harvard Medical School. He was succeeded as LI Chief by Paul.

In retrospect, Benacerraf's major contributions to NIAID involved the reorganization of the LI; realignment and focusing of staff interests; and introduction of studies on one of the most important problems of immunology of the day, cell-cell-interactions. These accomplishments set the stage for continued sophisticated research in immunobiology and immunogenetics at cellular and molecular levels. His influence was responsible for creating an intellectual environment which attracted many high quality research fellows to the LI, many of whom advanced to staff positions and in turn enhanced the impact of Benacerraf's relatively short stay at NIAID.

Laboratory of Germfree Animal Research (1959-1969)
Laboratory of Microbial Immunity (1969-72)

In 1959 the Laboratory of Germfree Animal Research (LGAR) was established as one of four NIAID laboratories. At the start and during the early years of its history, its investigations were devoted to the biologic aspects of experimental animals born and maintained in microbial free environments. In particular, the LGAR initially directed its concern to technical problems associated with keeping germ free animals alive. However, it was only to be expected that the potential of this facility for advancing immunologic research would soon be appreciated. Within the settings offered by the LGAR were unique opportunities to study the structure and function of immune systems that had escaped antigenic challenges of microbial agents and their products at varying periods from birth through senescence.

Within three years after its creation, an immunopathologist was added to the LGAR staff. In 1962, Edwin M. Lerner moved from his position in the National Institute of Arthritis and Metabolic Diseases to take up this NIAID appointment. In collaborations that crossed laboratory organizational lines, Lerner, with McMaster and Stone of the LI, conducted investigative studies on thyroiditis and experimental allergic encephalomyelitis.[42] The pace of immunologic investigations within LGAR, introduced by Lerner in 1962, soon picked up momentum. The following year, Richard M. Asofsky, after completing training in pathology and immunology at New York University, joined the Laboratory staff to begin his early studies on immunoglobulin synthesis.

[m] described in a following section on the Laboratory of Clinical Investigation

One year later, in 1964, after completing his obligation to serve as interim Acting Chief of the Laboratory of Immunology, Tobie moved to the LGAR to become its new Laboratory Chief. Also accompanying Tobie on transfer from the LI to LGAR appointment was McMaster. With this change in leadership and staff additions, immunology became the center of the Laboratory's research endeavors. During Tobie's tenure as LGAR Chief, he continued to pursue studies on the biology of the human immune response to malarial infection. McMaster's series of studies on EAE similarly were extended.[43]

Further realignments in staff associations and the character of investigations occurred. In 1965, Lerner transferred. to the LI to pursue his work in immunopathology. In 1968, Philip J. Baker, after completing a staff fellowship in the LI, joined the LGAR to further his investigational interests in microbial immunity, and Harvey M. Cantor came to NIH to work as a staff fellow with Asofsky.

Baker's studies centered on the induction of antibody responses and tolerance to microbial-derived polysaccharides. These lead to a seminal observation on the regulation of immune responses. In an analysis of T-independent responses to type III pneumococcal polysaccharide in mice, Baker independently demonstrated a new regulatory cell type, the suppressor T cell. Also, for the first time, the role of T cells in putative T-independent responses was documented.[44]

Asofsky and Cantor, in graft vs. host reaction (GvH) studies, were the first to describe T-T cell interactions demonstrating the synergistic actions within mixtures of young and old lymphoid cells in the induction of GVH.[45] This seminal finding set the stage for the identification of T cell populations involved in the augmentation and suppression of immune responses.

Because of the continuing change of focus in LGAR research activities, it was appropriately renamed the Laboratory of Microbial Immunity (LMI) in 1969. The following year, when Tobie was given additional NIAID-wide duties as Assistant Scientific Director of Laboratory and Clinical Research, Asofsky was named Assistant Chief of the LMI. Two years later, upon Tobie's retirement in 1972, Asofsky replaced him as Laboratory Chief.

Laboratory of Clinical Investigation (1952-1970)

The Laboratory of Clinical Investigation (LCI) dates its establishment to 1952, coincident with the opening of the NIH Clinical Center. Since, at that time, the founding of the Laboratory of Immunology by Freund was still six years away, two of NIAID's meritorious early entries into the arena of immunobiology and immunochemistry were launched and nurtured in the LCI. Later transfer into the more appropriate setting of the LI for basic studies became possible. An account of the LCI genesis of Sheldon Dray's studies on immunoelectrophoresis and rabbit immunoglobulin allotypy is contained in a preceding section on history of the LI. In a similar manner, LCI also offered the requisite base and resource for Rose Lieberman to initiate her work in a comparable area of research endeavor.

Lieberman's opportunity to work at NIH in the position of Microbiologist was of necessity delayed, pending the opening of the Clinical Center. During the three-year waiting period, she spent two years as a Medical Bacteriologist at the Dayton, Ohio, Veterans Administration Hospital, and a third as a Research Bacteriologist at the Ft. Detrick, Frederick facility of the U.S. Army Biological Corps. Joining the original LCI staff in 1952, Lieberman spent the first year on detail to the Clinical Center setting up a clinical microbiology laboratory.

Whereas Dray's pioneering studies on allotypy utilized rabbit immunoglobulin, Lieberman's contributions on allotypes had their origin in her work with mice. In initial protocols employing Freund's complete adjuvant in intraperitoneal routes of immunization to raise anti-staphylococcus antibodies, she was able to produce ascites[46] where the attempts by others were unsuccessful because of strain differences in the mouse models. Application of this system in various mouse strains with Dray's collaboration led to the identification of mouse allotypes.[47] In addition to the original plan to utilize ascites containing antibody for the allotype studies, an associated finding contributed to a whole new line of investigation. Recognition by Michael Potter of NCI that the BALB/c strain concurrently was developing plasma cell tumors led to fruitful collaborations among LCI, LI, and NCI investigators. As a result of the excitement generated by this new finding, Lieberman, Dray and Potter launched a long series of studies on allotypes, idiotypes, and the use of the plasmacytomas as a source of pure H chains.[48] With Benacerraf's arrival at NIH and his expressed special interest in this line of investigation, Lieberman transferred her position of Research Microbiologist to the LI where these studies were expanded.

Having served an important and valuable function in initiating research studies in immunobiology, immunochemistry and immunogenetics before the advent of the Laboratory of Immunology, the LCI awaited the appropriate staff and time to dedicate resources to clinically relevant immunologic investigations. Its first approach—establishing a Clinical Immunology Section in 1960 through Goodman's joint LI-LCI appointments and in providing opportunities for collaborative projects by Kuvin, Tobie, and other LCI-LI staff—were described in an earlier section of this account. Although the time was right for LCI to develop its own independent immunologically-relevant program, the requisite leadership awaited the advent of appropriate circumstances for the initiative to be taken by Sheldon M. Wolff.

Wolff's arrival at NIAID in 1960 as a clinical associate was linked by circumstance to the appointment of his mentor, Vernon Knight, at a senior level within NIAID. Association with Knight dated back to Wolff's training in internal medicine during the preceding few years at Vanderbilt University, where Knight had served on the faculty and hospital staff. In 1959, upon being named NIAID's second LCI Chief and NIAID Clinical Director to fill the position formally held by Norman McCullough,

Knight offered Wolff the opportunity to join him at NIH and to extend his interests to infectious diseases. Wolff's plans and career goals in endocrinology were then put aside.

Knight directed his energies and expertise to strengthening and expanding the LCI's program in infectious diseases. The initiation of clinical studies in immunology depended on developing intra-laboratory associations between LCI and LI. Wolff's first exposure to this new area of endeavor was fostered by the establishment of the joint LI-LCI Section, effected through the Knight-Tobie agreement, and a later opportunity to pursue cooperative studies when Landy replaced Tobie as LI Chief. With Goodman, Wolff conducted investigations on antimetabolite agents in immune system related diseases and with Landy, developed the beginnings of an extensive series of studies on endotoxin and fever.[49] These proved to be expanding areas that would engage his major research interests and expertise during subsequent years. However, with Goodman's taking up a new position with WHO and departure for Geneva in 1963 and the resultant disappearance of the joint Section on Clinical Immunology, these early active and vibrant LI-LCI associations in immunologic research began to fade. However, clinical immunology would reappear as an expanding LCI research endeavor when Wolff was presented with the opportunity a few years later.

Advancing within LCI through appointments as Senior Investigator and Head of the Clinical Immunology Section, Wolff was named Acting LCI Chief and Acting NIAID Clinical Director six years after his arrival, when Knight departed to take up the position of Chairman of the Department of Microbiology and Immunology at Baylor College of Medicine. Two years later, Wolff's positions were converted to permanent appointments, which he held until 1977, when he left to assume the Department of Medicine chairmanship at Tufts University.

The period of Wolff's tenure witnessed an escalation in the quality of research in immunology, achieving creditable recognition parallel to counterpart NIAID laboratories of basic immunology, microbiology, and infectious diseases and related developing activities at NCI and other Institutes. The mechanism of dual appointments with other NIAID intramural laboratories was utilized to further opportunities for interchanges of ideas and techniques in immunology and to encourage collaborative efforts. When Wolff took over as LCI Chief and NIAID Clinical Director, excellent virology and mycology services remained from previous tenures. However, there was not much more in the way of other strong efforts. Staff departures for academic opportunities had depleted personnel in all but two senior level positions for supervision of clinical investigations related to the 52 Clinical Center beds allotted to NIAID. Wolff then made the decision to interface immunology with infectious diseases through a new LCI program to examine adult host defense mechanisms. Accordingly, a highly selective recruitment process went forward for appropriately qualified staff associates and for fellows with potential for development as independent investigators. This initiative proved to be both successful and rewarding.

During the period through 1970 covered by this account, important original work at the forefront of research in clinical immunology was carried out by Wolff and clinical associates who advanced to LCI staff positions, e.g. Harry R. Kimball, John N. Sheagren, and Richard K. Root.[50] Their highly productive studies included studies on febrile tolerance, bacterial endotoxins, phagocytic functions, neutropenia, Chediak-Hygashi syndrome in man and animal models, experimental immune fever, and human leukocytic pyrogen.

Also during the period covered by this account through 1970, senior staff associates who had been recruited by Wolff to undertake investigations in clinically-relevant areas of immunology initiated their series of independent studies. This was only the beginning of Michael M. Frank's long association with LCI and his early research contributions to complement biology.[51] This proved to be an area of special research interest in which he would gain distinguished recognition. Frank, who joined NIAID in 1968 as Senior Investigator after leaving a position with the National Institute of Child Health and Human Development, subsequently succeeded Wolff as LCI Chief and NIAID Clinical Director in 1977. It was also the beginning for Charles H. Kirkpatrick, recruited in 1968 from the University of Kansas to develop a Section of Clinical Allergy and Hypersensitivity, and his series of studies on cell-mediated immunity and chronic mucocutaneous candidiasis.[52]

It is pertinent to note that, in the immediate years that followed, a number of LCI clinical associates who trained at NIAID with Wolff engaged in highly productive and rewarding endeavors in clinical immunology. Their important work placed LCI at the fore of studies on Wegener's granulomatosis and mid-line granuloma, the hypereosinophilic syndrome, Chediak-Hygashi syndrome, necrotizing vasculitis, granulomatous liver disease, cyclic neutropenia, neutrophil biology and phagocytic mechanisms, corticosteroids and cytotoxic immunosuppressive agents in the treatment of collagen vascular diseases, and immunoregulatory mechanisms. Among this group and remaining at NIAID was Anthony S. Fauci, who advanced to the position of NIAID Deputy Clinical Director in 1977, was named to head a newly-established, independent Laboratory of Immunoregulation in 1980, and succeeded Richard M. Krause as NIAID Director in 1984. Also, John I. Gallin, Head of the LCI Section on Bacterial Diseases, succeeded Kenneth Sell as Director of the NIAID Intramural Program in 1985.

Allergy

A period of 59 years elapsed from the time of Rosenau and Anderson's classic studies on anaphylaxis[2] before the designation *allergy* was incorporated in the name of an NIH component Institute in 1955. During this interval, the only recognition of allergic mechanisms as a subject for study was the work of Germuth on hypersensitivity in the Division of Infectious Diseases in the late 1940s-early 1950s.[11] Three years after the mission given to NIAID, studies on anaphylaxis appeared in experimental protocols of the newly-created LI with the resumption of Freund's and Stone's studies initiated earlier at the Public Health Research Institute of New York. It was still an additional four years before the name of allergy became associated with LI endeavor and a Section on Allergy and Hypersensitivity headed by Stone was created by Landy after replacing Freund as Laboratory Chief.

Meanwhile, two years earlier, the first indication of NIH's intent to serve the interests of human allergic disease appeared apart from NIAID in a new program within the NIH Division of Biologics Standards (DBS). In 1960, Harold Baer was recruited from the Department of Microbiology at Tulane University to take on the responsibilities of Chief in the newly-created Section on Allergenic Products in the DBS. Baer's early interests and studies in cell-mediated immunity and delayed-type hypersensitivity reactions to mycobacterial fractions, BCG and tuberculin, led him to consider the pressing nature of problems in human contact dermatitis. Just five years earlier, in 1955, the DBS forerunner, Biologics Control Division, was detached from NIAID and expanded to Division status within NIH. Had this development been otherwise, Baer's investigations on delayed contact sensitivity to various chemical sensitizers—especially concerned with the chemistry of catechols in poison ivy dermatitis—and his leadership in organizing an international effort for the standardization of allergenic extracts would have been effected under an NIAID aegis.

Baer's expertise, nevertheless, was made available to NIAID through interactions in a special project undertaken in 1959. At that time a collaborative group headed by the noted immunochemist, Dan H. Campbell of the California Institute of Technology, attacked the problem of characterization of allergens. As mentioned earlier, Campbell had been associated with NIAID intramural staff investigations when Vannier was detailed to work in his laboratory while awaiting the availability of intramural space after recruitment to the LI.[53] To initiate the work of Campbell's three subcommittees on chemistry, animal testing, and clinical trials, respectively, ragweed was chosen as the prototype for the fractionation, isolation and identification of allergenic fractions of etiologic importance in seasonal hay fever and asthma. Baer, in this cooperative DBS-NIAID effort, assumed the responsibility for quality control and supply of ragweed pollen derived-starting materials.

In any event, 15 years would go by after NIAID was established before the identity of a program on clinical allergy appeared within the LCI in 1968. There was, however, a near miss in the start-up of a possible program on clinical allergy in 1966. In Seal's search to fill Knight's vacated positions of LCI Chief and NIAID Clinical Director, an offer was made to John H. Vaughan of the University of Rochester. Vaughan, with credentials in allergy and clinical immunology, upon acceptance indicated that high on his list of priorities was the institution of broad scope and in-depth studies on asthma. Through periodic visits to Bethesda, he already had begun to discuss programs and explore a spectrum of possibilities with Institute staff. During this process, quite suddenly, Vaughan was faced with unexpected illness. After reassessing the uncertainties of his future, Vaughan withdrew. For the time being, the positions of LCI Chief and NIAID Clinical Director remained vacant, and the promise of an allergic disease program remained unfilled.

In building clinical immunology within the LCI, Wolff was alert to the Institute's need to put a program in clinical allergy in place. Additionally pertinent, Davis as Institute Director and Seal as Scientific Director from the beginning were strongly supportive of this objective. Wolff in his search was successful in recruiting Charles H. Kirkpatrick from the faculty of the University of Kansas. As the newly-appointed Head of a Section of Allergy and Hypersensitivity in 1968, Kirkpatrick planned to establish a program on the pathogenesis of asthma, extending his earlier preliminary studies on autonomic nervous system responses in patients with various types of asthma, and to evaluate certain other allergic diseases. Upon arrival at NIAID, three items engaged his attention.

The first was an issue quite easily resolved. In order to avoid duplication of name and bypass confusion with the LI Section on Allergy and Hypersensitivity, with Stone's agreement, the LCI's Section was renamed Clinical Allergy and Hypersensitivity. Second, Kirkpatrick noted that only one other scientist at NIH, Harold Baer, was really interested in allergic disease and allergic mechanisms. Contact was initiated and led to a Baer-Kirkpatrick collaboration in a study of synthesized pentadecyl catechols of varying structure in sensitization to poison ivy.[54]

The third was an unanticipated stumbling block encountered in attempting to get an asthma program underway—the absence of available equipment for pulmonary function studies. Some fairly sophisticated instrumentation did exist in the National Heart and Lung Institute. However, to his chagrin, Kirkpatrick was advised that the NHLBI pulmonary function equipment could not be made available for NIAID use in a program on asthma. There were two alternatives. One, requisite equipment could be purchased, but the necessity of converting critical space allotted to a research laboratory into a pulmonary function laboratory hampered this move. The second option, in which patients were sent to the National Naval Medical Center for pulmonary function studies, did not really answer the problem. As a result, the program sputtered and never really realized its promise.

A turning point in the direction of studies occurred about 1970 when, on the basis of earlier observations and experience in Kansas, the opportunity was presented for Kirkpatrick to establish a protocol on immunologic abnormalities in T cell functions associated with chronic mucocutaneous candidiasis.[52] This line of investigation also provided an opportunity for Kirkpatrick to initiate studies on therapeutic applications of leucocyte-derived material known as transfer factor in attempting to compensate for an immune system defect.[55]

Although Kirkpatrick's scientific interests were being diverted to problems of human deficiency disorder in cell-mediated immunity, there was no loss of interest in developing a program on the classic allergic or atopic diseases. Only temporarily diverted, plans for creating a new section on Allergic Diseases reached fruition. With the recruitment of Allan M. Kaplan from the Brigham Hospitals-Harvard University Medical Center to head the Section in 1972, studies got underway on molecular mechanisms in the activation of vasoactive inflammatory mediators and syndromes of urticaria and angioedema. When Kaplan was joined in 1975 by his former Brigham-Harvard associate, Michael A. Kaliner, NIAID investigations on asthma appeared among LCI protocols with studies on autonomic nervous system abnormalities in patients with upper and lower respiratory tract allergic diseases. At long last, research programs truly reflecting the implication of the descriptor *allergy* in NIAID's name had reached fruition.

The Rocky Mountain Laboratory (1950-1970)

In the years following the establishment of the Rocky Mountain Spotted Fever Laboratory in 1921 as a Public Health Service field station and its later incorporation into the NIH Division of Infectious Diseases as the Rocky Mountain Laboratory (RML) in 1937, the primary thrust of its research program was in the area of microbiology and infectious diseases. Thus, any immunologic relevance of its initial endeavors was in the field of microbial immunity and, later during the World War II years, in the development of vaccines. Following the incorporation of the Division of Infectious Diseases into the newly-created National Microbiological Institute, RML programs were influenced by the mission of its new parent Institute.

In seeking new leadership to rejuvenate and expand the RML, the Institute identified Carl M. Larson, who had joined the NIH Division of Infectious Diseases in 1939, after holding a faculty appointment at George Washingron University. Appointed RML Director iu 1950, Larson was sensitive to the need to include immunologic research endeavors in redefining the Laboratory's mission. Thus, in initiating recruitment from NMI staff, especially from within the Laboratory of Infectious Diseases and the Laboratory of Biologics Control, Larson invited Samuel B. Salvin and Edgar Ribi to join him at RML. Through their combined interests, the stage was set for RML's expansion in the new directions of immunology and cell biology.

To join NIH, in 1946, all that Salvin had to do was cross the street from the US Naval Medical Center in Bethesda. A background in mycology led to his recruitment to the NIH Division of Infectious Diseases, especially to study the non-mycobacterial etiologic agent of pulmonary lesions that roentgenographically stimulated tuberculosis. In working with *Histoplasma capsulatum* Salvin was able to recover specific antigen from intact cultured cells that was capable of detecting complement-fixing antibodies. This property contrasted with the lack of antigenic efficacy of the broth filtrate preparation histoplasmin in serologic procedures. Salvin's contribution provided the method for correlation of laboratory *in vitro* testing with clinical status. At DID and its successor, the NMI Laboratory of Infectious Diseases, he continued to pursue his research interests on pathogenic fungi.[56] During the thirteen years that Salvin spent at RML, he published extensively in the area of fungal immunity; his studies proved to be important in revealing and characterizing mechanisms of delayed-type hypersensitivity and the role of cell-mediated immunity in the control of infection.[57] In 1964, Salvin left the RML to accept a position with CIBA Pharmaceutical Corporation as Head of its Immunology Research Division.

Another important recruit was Edgar Ribi who also accompanied Larson to RML. Uppsala University in Sweden provided the setting for the crossing of paths between Charles C. Shepard and Ribi. At that time, Ribi was a research associate in physical chemistry; Shepard, an NMI bacteriologist, was spending a year in residence at the University. As a result of their interactions, Ribi was impressed with the possible opportunities to further his career at NIH and accepted a position as a fellow in 1952. After a relatively short stay, he was recruited by Larson.

Ribi's subsequent studies and development of special expertise led to his international recognition for work in defining the composition of bacterial endotoxins and the cellular components of *Mycobacterium tuberculosis* responsible for induction of immune responses to this microbial infectious agent. The nature of these studies on mycobacteria involved collaborative efforts with other RML scientists, including Larson and Robert L. Anacker.[58,59] Another of his contributions was the technical development of the Ribi Cell Fractionator. This unique laboratory aid, which facilitated the isolation of bacterial cell components, subsequently turned Ribi's attention to adjuvant properties of mycobacterial cell walls and application of these bacterial products to immunotherapy in cancer.[60] In 1980, Ribi retired from his position at RML and remained in Hamilton, Montana to found Ribi Immunochem Research and head its laboratories.

As a consequence of Larson's continuing efforts to build immunology at RML, John J. Munoz was recruited to the RML staff in 1961. Munoz was introduced to the NIAID through the proximity of RML to the nearby University of Montana where he was serving as Chairman of the Department of Microbiology and Public Health and Director of the Stella Duncan Memorial Laboratories. His area of research interests through that time had included studies on the fractionation, and biochemical and immunologic properties of bacterial antigens, vaccine development, and related hypersensitivity

mechanisms. As Chief of the RML Allergy and Immunology Section, Munoz's studies in the field of microbial immunity led to important contributions on the *Bordetella pertussis* organism. Included in this series were investigations on purification, identification and mechanisms of action of various *B. pertussis* protective antigenic fractions[61] and histamine sensitizing factors (pertussigen).[62] From allergy's standpoint, this line of immunologic investigation bore direct relevance to IgE mechanisms, to the production of allergic encephalomyelitis, and to the problem of adverse reactions to pertussis vaccine in childhood immunization.

After becoming Director of RML in 1964, Herbert G. Stoenner reinforced the Laboratory's continuing interest in immunology with the appointment of John E. Coe in 1965. Coming from a fellowship in immunopathology at Scripps Clinic and Research Foundation, Coe was quite familiar with the RML; he had spent three years (1960-63) at the NIAID Montana facility working with Salvin on mechanisms of delayed-type hypersensitivity. Coe's early studies in his RML appointment focused on the characterization of humoral immune responses in several species of rodents, especially the Syrian hamster.[63]

RML's historical development is an intriguing story of the evolution of a spectrum of microbiologic research as an outgrowth of public health concern for a single disease entity. Additionally, it is representative of NIAID's growth during the period of classic bacteriology's transition to present day cellular and molecular biology and virology. To Larson, credit is due not only for guiding RML's progress in its original, defined mission, but also for recognizing the need for an immunologic component as a vital interdisciplinary force. In retrospect, through the insights of Larson's original group, full advantage was taken of unique Institute interests and resources, which contributed in a very special way to a noteworthy NIAID immunologic endeavor—microbial immunity.

Comment

This account of the development of immunology at the Laboratory of Hygiene/Hygienic Laboratory (1887-1930), NIH (1930-1948), NMI (1948-1955), and NIAID (1955-circa 1970) in a way presents only the beginning of an endeavor which the Institute has just reason to view with pride and sense of reward. The continuing story of events and assessments in the years that followed belong to the current group of concerned Laboratory Chiefs, whose vibrant activities, accomplishments, and goals are very much a part of today's intramural scene. We expect that their tasks soliciting contributions for highlighting from the many made by staff and programs under their respective purviews will be considerably more difficult than ours has been.

It is unlikely that even the most visionary of the medical scientists, mentioned in this account of NIH's first 83 years, could have envisioned the escalating pace at which basic research data have been generated—a phenomenon largely responsible for immunology's remarkable advances and for the creation of an array of biotechnologies that have appeared almost explosively in so short a time. Nor is it expected that even the most insightful clinicians of the period could have foreseen how immunology would become so relevant to an understanding of mechanisms in a good number of diverse system diseases and its great potential for the application of this unfolding new knowledge to the development of improved methods for their diagnosis, treatment, and prevention.

Yet, in this centennial year, we would be insensitive to the historical implications and remiss in acknowledging appreciation to those who made it all happen; to those who set the stage for future inventiveness and inquiry, and to those whose contributions to the Institute also provided the foundation upon which modern-day immunologic scientists continue to build in both theoretical and practical fashion surprising to even the keenest imagination.

Note Regarding the Naming of NIAID:

For reasons and events that led to the change in name from the National Microbiological Institute to the National Institute of Allergy and Infectious Diseases proposed by concerned NIH officials, the authors are grateful to Victor H. Haas who served as NMI and NIAID Director during that period. In providing this information, Haas recalled the following incident.[b]

"Shortly after I began my assignment as Director of the Microbiological Institute, (1948) the NIH amateur theatrical group known as the 'Hamsters' presented its annual production of skits lampooning events and people at NIH. One of the skits centered on the Microbiological Institute. Its theme was that the Institute was a sort of orphan, an amorphous creation among the richer and more 'glamorous' categorical Institutes, e.g. NCI, NHI, NIMH. The players rendered a musical plea for improving the Institute's status with the following doggerel, among others

'Lenny, please Lenny
Won't you categorize me tonight?
Lenny, Don't you love me any,
Please categorize me tonight'

'Lenny' referred to Leonard Scheele, the Surgeon General"

Acknowledgments

In the search and gathering of material for this account, the authors took advantage of the opportunity offered for inquiry and discussion with several current and past members of the Institute's scientific and professional staff. We gratefully acknowledge their thoughtful consideration and many courtesies extended to us, and the pleasantries of time spent in reflections and remembrances.

We especially appreciated the availability, cordial welcomes, and valued assistance of those to whom we repeatedly turned as special resources to amplify and clarify specific points of information and to explore leads uncovered in various Institute files and documents of earlier years pertaining to periods of their involvements: Dorland J. Davis for the span of his tenures in NIH intramural positions and as NIAID Director, Victor H. Haas for the NMI-NIAID transitional period, Sanford H. Stone for LI, Howard C. Goodman for LI and LCI, John E. Tobie for LI and LGAR, Maurice Landy for LI, Richard M. Asofsky for LMI, Baruj Benacerraf for LI, Sheldon M. Wolff for LCI, Charles H. Kirkpatrick for LCI/Allergy, and Herbert Stoenner and John J. Munoz for RML.

One of us (SGC) remembers with appreciation the many pleasant hours spent with John R. Seal during the part of his lifetime that our respective tenures in NIAID staff positions overlapped. Just as in the case of current association with Dorland Davis, there were spin-off benefits in addition to the rewards of warm friendship. John Seal's reminiscences in conversation provided a wealth of information on a panorama of NIH/NIAID events used as background material in this account. Vivid memories of associates recall the strength of judicious leadership and support he provided in furthering the development of NIAID intramural programs in immunology and allergy.

With much appreciation, the authors also greatly acknowledge and thank Carol K. Shapiro for valued editorial assistance and dedication to the task of intensive preparation of manuscript material.

Sheldon G. Cohen, M.D.

Dr. Cohen initially joined NIAID in 1972 as a full-time consultant in a special project for the Extramural Programs. The following year he moved into an Extramural Staff position as Chief of the Allergy and Immunology Branch, and with the NIAID reorganization in 1977 was selected for the newly created position of Director of Immunology, Allergic and Immunologic Diseases Program. Dr. Cohen was born in Pittston, Pennsylvania in 1918, graduated from Ohio State University in 1940, and received his M.D. from New York University in 1943. After internship at New York's Bellevue Hospital he served as a flight surgeon with the U.S. Army Air Force in the European Theatre, and subsequently completed residencies in internal medicine and allergy and a research fellowship in applied physiology and immunology at the University of Pittsburgh. From 1952-1972 he pursued his career in the Wilkes-Barre, Pennsylvania area combining clinical practice and academic and research interests at Wilkes College in experimental biology and immunology and in affiliation with the Veterans Administration Hospital. Dr. Cohen has an extensive list of publications reporting on original studies in allergy and immunology, and during more recent years has published on subjects dealing with the history of medicine—an interest developed during his tenure as Historian of the American Academy of Allergy from 1963 to 1969.

William Raymond Duncan, Ph.D.

Dr. Duncan joined the NIH during its Centennial year as a Health Science Administrator in the Immunology, Allergic and Immunologic Diseases Program. Born in Harlingen, Texas in 1949, he graduated from the University of Texas at Austin in 1971 with a B.A. In 1976 he obtained a Ph.D. in Cell Biology from the University of Texas Health Science Center at Dallas. There he also held two postdoctoral fellowships in cancer immunology and a faculty position in the Department of Cell Biology. From 1983 to 1987 Dr. Duncan served on the faculty of Dalhousie University in Halifax, Nova Scotia.

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Biographical Notes

ANACKER, Robert Leroy (1926-); b. Minneapolis, Minnesota; Ph.D., University of Washington, 1956.

ANDERSON, John Fleetezelle (1873-1958); b. Fredericksburg, Virignia; M.D., University of Virginia, 1896.

ASOFSKY, Richard Marcy (1933-); b. New York, New York; M.D., State University, New York, 1958.

BAER, Harold (1918-); b. New York, New York; Ph.D., Harvard University, 1942.

BAKER, Philip John (1935-); East Chicago, Indiana; Ph.D., University of Wisconsin, 1960.

BENACERRAF, Baruj (1920-); b. Caracus, Venezuela; M.D., Medical College of Virginia, 1945.

BENGTSON, Ida Albertina (1881-1952); b. Harvard, Nebraska; Ph.D., University of Chicago, 1919.

BOZICEVICH, John (1905-); b. Sunnyside, Utah; M.A., George Washington University, 1930.

CAMPBELL, Dan Hampton (1908-); b. Freemont, Ohio; Ph.D., Washington University, 1935.

CANTOR, Harvey (1942-); b. New York, New York; M.D., New York University, 1967.

COE, John Emmons (1931-); b. Evanston, Illinois; M.D., Hahnemann Medical College, 1957.

DAVIS, Dorland Jones (1911-); b. Chicago, Illinois; M.D., Johns Hopkins University, 1937; D.P.H., Johns Hopkins University, 1940.

DRAY, Sheldon (1920-); b. Chicago, Illinois; M.D., University of Minnesota, 1946; Ph.D., University of Minnesota, 1954.

DYER, Rolla Eugene (1886-1971); b. Delaware County, Ohio; M.D., University of Texas, 1915; Sc.D., Washington University, St. Louis, 1916.

FAHEY, John Leslie (1924-); b. Cleveland, Ohio; M.D., Harvard University, 1951.

FAUCI, Anthony Stephen (1940-); b. Brooklyn, New York; M.D., Cornell University, 1966.

FELTON, Lloyd Derr (1885-1953); b. Pine Grove Mills, Pennsylvania; M.D., Johns Hopkins University, 1916.

FRANK, Michael M. (1937-); b. Brooklyn, New York; M.D., Harvard University, 1960.

FREUND, Jules (1890-1960); b. Budapest, Hungary; M.D., University of Budapest, 1913.

GALLIN, John Isaac (1943-); b. New York, New York; M.D., Cornell University, 1969.

GERMUTH, Frederick George, Jr. (1921-1984); b. Baltimore, Maryland; M.D., Johns Hopkins University, 1945.

GOLDBERGER, Joseph (1874-1929); b. Austria-Hungary; M.D., Bellevue Hospital Medical College (New York University), 1895.

GOODMAN, Howard Charles (1920-); b. Rochester, New York; M.D., Johns Hopkins University, 1944.

GREEN, Ira (1926-); b. New York, New York; M.D., State University of New York at Brooklyn, 1953.

HABEL, Karl (1908-1981); b. Philadelphia, Pennsylvania; M.D., Jefferson Medical College, Philadelphia, 1933.

INMAN, John Keith (1928-); b. St. Louis, Missouri; Ph.D., Harvard University, 1956.

KAYHOE, Donald Ellsworth (1920-); b. Washington, D.C.; M.D., George Washington University, 1950.

KIMBALL, Harry Raymond (1937-); b. Los Angeles, California; M.D., University of Washington, 1962.

KINYOUN, Joseph James (1860-1919); b. East Bend, North Carolina; M.D., New York University, 1882.

KIRKPATRICK, Charles Harvey (1931-); b. Topeka, Kansas; M.D., University of Kansas, 1958.

KNIGHT, Vernon (1917-); b. Osceola, Missouri; M.D., Harvard University, 1943.

KUVIN, Sanford Bernard (1929-); b. Newark, New Jersey; M.D., Cambridge University, England, 1957; D.T.M.H., University of London, 1963.

LANDY, Maurice (1913-); b. Cleveland, Ohio; Ph.D., Ohio State University, 1940.

LARSON, Carl Leonard (1909-1978); b. Butte, Montana; M.D., University of Minnesota, 1939.

LERNER, Edwin M. (1919-); b. Ottawa, Ontario; M.D., Harvard University, 1944.

LIEBERMAN, Rose (1912-); b. New York, New York; M.A., Columbia University, 1937.

MAGE, Rose G. (1935-); b. New York, New York; Ph.D., Columbia University, 1963.

MAXCY, Kenneth Fuller (1889-1966); b. Saco, Maine; M.D., Johns Hopkins University, 1915; D.P.H., Johns Hopkins University, 1921.

McMASTER, Philip Robert Bache (1930-); b. Cambridge, Massachusetts; M.D., Johns Hopkins University, 1956.

MUNOZ, John Joaquin (1918-); b. Guatemala; Ph.D., University of Wisconsin, 1947.

PATERSON, Philip Y. (1925-); b. Minneapolis, Minnesota; M.D., University of Minnesota,

PAUL, William Erwin (1936-); b. Brooklyn, New York; M.D., State University of New York, Downstate Medical Center, 1960.

REISFELD, Ralph Alfred (1926-); b. Stuttgart, Germany; Ph.D., Ohio State University, 1957.

RIBI, Edgar (1920-1987); b. Zurich, Switzerland; Ph.D., Berne University, 1948.

RICH, Robert Reiger (1941-); b. Newton, Kansas; M.D., University of Kansas, 1966.

ROOT, Richard Kay (1937-); b. New York, New York; M.D., Johns Hopkins University, 1963.

ROSENAU, Milton Joseph (1869-1946); b. Philadelphia, Pennsylvania; M.D., University of Pennsylvania, 1889.

SALVIN, Samuel Bernard (1915-); b. Boston, Massachusetts; Ph.D., Harvard University, 1941.

SCHULTZ, William Henry (1873-1947); b. Canal Fulton, Ohio; Ph.D., Johns Hopkins University, 1907.

SEAL, John Ridley (1912-1984); b. Charleston, West Virginia; M.D., University of Virginia, 1937.

SHEAGREN, John Newcomb (1935-); b. Aurora, Illinois; M.D., Columbia University, 1962.

SHEVACH, Ethan Menahem (1943-); b. Brookline, Massachusetts; M.D., Boston University, 1967.

SMADEL, Joseph E. (1907-1963); b. Vincennes, Indiana; M.D., Washington University, 1931.

STONE, Sanford Herbert (1921-); b., New York, New York; Sc.D., University of Paris, 1951.

STRAUSS, Arthur (1933-); b. New York, New York; M.D., Columbia University, 1958.

TOBIE, John Edwin (1911-); b. Collison, Illinois; Ph.D., Tulane University, 1940.

VANNIER, Wilton Emile (1924-); b. Pasadena, California; M.D., University of California, San Francisco, 1948; Ph.D., California Institute of Technology, 1958.

VAUGHAN, John Heath (1921-); b. Richmond, Virginia, M.D, Harvard University, 1945.

WILLIAMS, Alvin Curtis, Jr. (1927-); b. Moorestown, New Jersey; Ph.D., Rutgers University, 1954.

WOLFF, Sheldon Malcolm (1930-); b. Newark, New Jersey; M.D., Vanderbilt University, 1957.

Part II
Intramural Contributions, 1971-present

Laboratory of Biology of Viruses (LBV)

Established: 1959



Chief: Norman P. Salzman, Ph.D.
1968-86

Prior:



Karl Habel, M.D.
1959-67

History:
The laboratory was established in 1959 from the nucleus of the Basic Studies Section of the Laboratory of Infectious Diseases. In 1961 the former Laboratory of Cell Biology was incorporated into the structure of the Laboratory of Biology of Viruses as the Cell Biology Section. The laboratory was disbanded in 1986 when Dr. Salzman retired.

1971.
Gelb, Lawrence D.; Kohne, David E.; and Martin, Malcolm A. Quotation on Simian Virus 40 Sequences in African Green Monkey Mouse and Virus-Transformed Cell Genomes. *Journal Molecular Biology* 57: 129-45.

☐ This paper provided a method to quantitate the number of integrated DNA molecules present in transformed cells. It was a critical paper at the time that viral transformation was first being analyzed.

1971.
Sebring, Edwin D.; Kelly, Thomas J. Jr.; Thoren, Marilyn; and Salzman, Norman P. Structure of Replicating Simian Virus 40 Deoxyribonucleic Acid Molecules. *Journal of Virology* 8: 478-90.

☐ A description of the mechanism of replication of simian virus 40. It identified unique features contained in circular DNA molecules during DNA replication.

1972.
Fareed, George C.; Garon, Claude F.; and Salzman, Norman P. Origin and Direction of Simian Virus 40 Deoxyribonucleic Acid Replication. *Journal of Virology* 10: 484.

☐ This study established that there was a single specific origin for SV40 DNA replication and that DNA synthesis then proceeded in a bi-directional way.

1972.
Garon, Claude F.; Berry, Karen W.; and Rose, James A. A Unique Form of Terminal Redundancy in Adenovirus DNA Molecules. *Proceedings of the National Academy of Sciences, USA* 69: 2391-95.

☐ This study was the first description of inverted terminal DNA repeats that were important in the mchanism of viral DNA replication.

1974.
Wei, C. M., and Moss, Bernard. Methylation of Newly Synthesized Messenger RNA by an Enzyme in Vaccinia Virus. *Proceedings of the National Academy of Sciences, USA* 71: 3014-18.

☐ First in a series of papers which describe enyzmes that cap viral and cellular messenger RNAs.

1975.
Martin, S. A.; Paoletti, E.; and Moss, Bernard. Purification of mRNA Guanylytranserase and mRNA(guanine-7-)methyltransferase from Vaccinia Virions. *Journal of Biological Chemistry* 250: 9322-29.

☐ First isolation of capping enzyme.

1975.
Wei, C. M.; Gershowitz, A.; and Moss, Bernard. Methylated Nucleotides Block 5' Terminus of HeLa Cell Messenger RNA. *Cell* 4: 379-86.

☐ First demonstration of cap structure of cellular mRNA.

1976.
Straus, Steven E.; Sebring, Edwin D.; and Rose, James A. Concatamers of Alternating Plus and Minus Strands are Intermediates in Adenovirus-Associated Virus DNA Synthesis. *Proceedings of the National Academy of Sciences, USA* 73: 742.

☐ The first *in vivo* description of the scheme of self primed DNA replication mechanism.

1982.
Baroudy, B. M.; Venkatesan, S.; and Moss, Bernard. Incompletely Base-Paired Flip-Flop Terminal Loops Link the Two DNA Strands of the Vaccinia Virus Genome into One Uninterrupted Polynucleotide Chain. *Cell* 28: 315-24.

☐ First demonstration of unique hairpin structure at the end of a linear DNA molecule.

1983.
Smith, G. L.; Mackett, M.; and Moss, Bernard. Infectious Vaccinia Virus Recombinants that Express Hepatitis B Virus Surface Antigen. *Nature* 302: 490-5.

☐ First demonstration of potential of vaccinia virus to make live recombinant vaccines.

1987.
Natarajan, Venkatachelos; Madden, Mary Jane; and Salzman, Norman P. Identification of a Transcription Factor which Interacts with the Distal Domain of the Adenovirus IVa2 Promoter. *Journal of Virology* 61: 646-52.

☐ Gene regulation of two adjacent genes was shown to be regulated by an identical protein binding to a shared regulatory DNA sequence.

Laboratory of Cellular and Molecular Immunology (LCMI)

Established: 1987



Chief: Ronald H. Schwartz, M.D., Ph.D.
1987-

History:
The Laboratory of Cellular and Molecular Immunology was founded in order to develop an integrated cellular and molecular approach to immunological problems related to T lymphocyte function, in particular T cell differentiation and T cell activation.

1978.
Schwartz, Ronald H.; Yano, A.; and Paul, William E. Interaction between Antigen-Presenting Cells and Primed T Lymphocytes: An Assessment of Ir Gene Expression in the Antigen-Presenting Cell. *Immunological Reviews* 40: 153-80.

☐ Summarizes the experiments with mice which demonstrates that Immune Response genes of the major histocompatibility complex are functionally expressed in antigen-presenting cells of the immune system such as macrophages and dendritic cells.

1985.
Schwartz, Ronald H. T Lymphocyte Recognition of Antigen in Association with Gene Products of the Major Histocompatibility Complex. *Annual Review of Immunology* 3: 239-63.

☐ Summarizes the experiments arguing in favor of the trimolecular complex model of T lymphocyte antigen recognition, where antigen is postulated to physically interact with the histocompatibility molecule.

Laboratory of Clinical Investigation (LCI)

Established: A clinical operation was contemplated or established in late 1950. Apparently first designated as the Clinical Investigation Branch the name of the laboratory (LCI) was established about March 1953.



Chief: Michael M. Frank, M.D.
1977-

Prior:



Sheldon M. Wolff, M.D.
1966-77



Vernon Knight, M.D.
1959-65



Norman B. McCullough, Ph.D., M.D.
1952-58

History:
The establishment of patient facilities in the Clinical Center as an adjunct to the research operation brought the National Institutes of Health full circle, since they had originated in 1887 as a small research laboratory attached to a patient facility (Marine Hospital, Staten Island, New York).

An official organizational chart of November 22, 1950, showed the laboratory at branch level with four authorized positions. In August 1952, the laboratory had a staff of eight. During these early years plans were being made for utilization of patient facilities in the new Clinical Center soon to be opened. A staff of 18 made the move to the Clinical Center in July 1953.

The laboratory has grown considerably and currently it is composed of several sections in which a variety of allergic, immunologic and infectious diseases are studied.

1971.
Frank, Michael M.; May, Joseph; Gaither, Thelma. A.; and Ellman, Leonard. *In Vitro* Studies of Complement Function in Sera of C4-Deficient Guinea Pigs. *Journal of Experimental Medicine* 134: 176-90.

☐ This first description of the biological properties of a new strain of guinea pigs genetically totally lacking in the complement protein C4. The animals were first described from NIAID by Drs. Leonard Ellman, Michael Frank and Ira Green. These were the first animals to be described lacking activity of the classical complement pathway and subsequently were the subject of hundreds of reports examining biochemistry, biology and molecular biology of complement proteins.

1972.
Wolff, S. M.; Dale, D. C.; Clark, R. A.; Root, R. K.; and Kimball, H. R. The Chediak-Higashi Syndrome: Studies of Host Defenses. *Annals of Internal Medicine* 76: 293-306.

☐ First review of abnormal host defense in Chediak-Higashi syndrome. Based on studies done at NIAID.

1973.
Clark, R. A.; Root, R. K.; Kimball, H. R.; and Kirkpatrick, C. H. Defective Neutrophil Chemotaxis and Cellular Immunity in a Child with Recurrent Infections. *Annals of Internal Medicine* 78: 515-22.

☐ First description of defective chemotaxis in the hyperimmunoglobulin-E recurrent infection syndrome.

1973.
Gallin, J. I.; Clark, R. A.; and Kimball, H. R. Granulocyte Chemotaxis: An Improved *In Vitro* Assay Employing ^{51}Cr Labeled Granulocyte. *Journal of Immunology* 110: 223-40.

☐ First automated assay of leukocyte locomotion. Widely used in 1970s and 1980s.

1973.
Lipsky, P. E., and Rosenthal, A. S. Macrophage-Lymphocyte Interaction. I. Characteristics of the Antigen-Independent-Binding of Guinea Pig Thymocytes and Lymphocytes to Syngeneic Macrophages. *Journal of Experimental Medicine* 138: 900-24.

☐ This was the first in a series of important papers describing the interaction of lymphocytes with macrophages and later the importance of macrophages in antigen presentation and the importance of histocompatibility type in allowing macrophages to present antigen to lymphocytes.

1973.
Rosenthal, A. S., and Shevach, E. M. The Function of Macrophages in Antigen Recognition by Guinea Pig T Lymphocytes. I. Requirement for Histocompatible Macrophages and Lymphocytes. *Journal of Experimental Medicine* 138: 1194-1212.

1973.
Rosenthal, A. S.; Rosenstreich, D. L.; Blake, J. T.; Lipsky, P. E.; and Waldron, J. A. Mechanisms of Antigen Recognition by T Lymphocytes. In F. Daguillard (Ed.), *Proceedings of the Seventh Leukocyte Culture Conference*. New York, Academic Press, Inc. pp. 201-206.

1973.
Rosenstreich, D. L., and Rosenthal, A. S. The Peritoneal Exudate Lymphocytes. II. *In vitro* Proliferation After Brief Exposure to Antigen. *Journal of Immunology* 110: 934-42.

☐ The research reflected in the three preceding publications was focused on defining the role of cell-cell interaction in macrophage-dependent activation of T-lymphocyte response to antigens; the role and location of immune response gene control of immunity and the mechanism of antigen processing and presentation via Ia molecule involvement in protein antigen recognition.

1974.
Gallin, J. I., and Rosenthal, A. S. The Regulatory Role of Divalent Cations in Human Granulocyte Chemotaxis. Evidence for an Association Between Calcium Exchanges and Microtubule Assembly. *Journal of Cell Biology* 62: 594-609.

☐ Initial studies of calcium metabolism and microtubule assembly in neutrophils exposed to chemoattractants. First demonstration of calcium release by chemoattractants.

1975.
Gallin E. K.; Wiederhold, M. L.; Lipsky, P. E.; and Rosenthal, A. S. Spontaneous and Induced Membrane Hyperpolarizations in Macrophages. *Journal of Cellular Physiology* 86: 653-62.

☐ First study of membrane potential in macrophages.

1975.
Lipksy, P. E., and Rosenthal, A. S. Macrophage-Lymphocyte Interaction. II. Antigen-Mediated Physical Interactions Between Immune Guinea Pig Lymph Node Lymphocytes and Syngeneic Macrophages. *Journal of Experimental Medicine* 141: 138-54.

1975.
Greineder, D. K., and Rosenthal, A. S. The Requirement for Macrophage-Lymphocyte Interaction in T Lymphocyte Proliferation Induced by Generation of Aldehydes on Cell Membranes. *Journal of Immunology* 115: 932-38.

1976.
Frank, Michael M.; Gelfand, Jeffrey A.; and Atkinson, John P. Hereditary Angioedema: The Clinical Syndrome Its Management. *Annals of Internal Medicine* 84: 580-93.

☐ This paper represents the first major clinical review of this syndrome and provided much of the early basis for the rational development of therapy of this disorder.

1976.
Gelfand, Jeffery A.; Sherins, Richard J.; Alling, David W.; and Frank, Michael M. Treatment of Hereditary Angioedema with Danazol: Reversal of Clinical and Biochemical Abnormalities. *New England Journal of Medicine* 295: 1444-48.

☐ This double blind study presented the "cure" of this genetically controlled disease by the use of a particular androgen. The genetically controlled, partial deficiency of a protein was actually reversed by therapy and the reduced levels of protein returned to normal in these patients. This became a mainstay of therapy in these individuals.

1976.
Kaliner, M. A. The Cholinergic Nervous System and Immediate Hypersensitivity. I. Eccrine Sweat Responses in Allergic Patients. *Journal of Allergy and Clinical Immunology* 58: 308-15.

☐ This article demonstrated for the first time that allergic subjects have hyperreactivity of the cholinergic nervous system and laid the basis for subsequent studies which evaluated the autonomic responsiveness of patients with allergic diseases in addition to asthma. This study was the first study to demonstrate that patients with allergic rhinitis or even patients who had any allergic disease had systemic abnormalities of autonomic responsiveness.

1977.
Atkinson, John P.; Waldman, Thomas A.; Stein, Sidney F.; Gelfand, Jeffery A.; MacDonald, Walter J.; Heck, Louis W.; Cohen, Edwin, L.; *et al.* Systemic Capillary Leak Syndrome and Monoclonal IgG Gammopathy: Studies in a Sixth Patient and a Review of the Literature. *Medicine* 36: 225-39.

☐ This is the first clinical description of this unusual syndrome in which patients lose the integrity of their post capillary venules and thus their intravascular volume. The disease was shown to be periodic and associated with the presence of a monoclonal immunoglobulin. This allowed for the recognition and treatment of other patients with this syndrome who formerly died.

1977.
Frank, Michael M.; Schreiber, Alan D.; Atkinson, John P.; and Jaffe, Charles J. Pathophysiology of Immune Hemolytic Anemia. *Annals of Internal Medicine* 87: 210-22.

☐ This review summarizes a series of papers presented in the *Journal of Clinical Investigation* which provided an explanation for the events that occur when an immunologically damaged erythrocyte enters the circulation. These papers provide an explanation for the pathophysiological basis of warm antibody (IgG) mediated autoimmune hemolytic anemia and cold agglutinin disease.

1977.
Gallin, E. K., and Gallin, J. I. Interaction of Chemotactic Factors with Human Macrophages: Induction of Transmembrane Potential Changes. *Journal of Cell Biology* 75: 277-89.

☐ First demonstration that chemotactic factors initiate changes in phagocyte membrane potential.

1977.
Malech, H. L.; Root, R. K.; and Gallin, J. I. Structural Analysis of Human Neutrophile Migration: Centriole, Microtubule and Micro Filament Orientation and Function During Chemotaxis. *Journal of Cell Biology* 75: 666-93.

☐ Demonstration of the importance of the cytoskeleton in neutrophil motility and demonstration of the critical role of the centriole in determining direction of cell movement.

1977.
Rosenthal, A. S.; Barcinski, M. A.; and Blake, J. T. Determinant Selection: A Macrophage Dependent Immune Response Gene Function. *Nature* 267: 156-58

1977.
Barcinski, M. A., and Rosenthal, A. S. Immune Response Gene Control of Determinant Selection. I. Intramolecular Mapping of the Immunogenic Sites on Insulin Recognized by Guinea Pig T and B Cells. *Journal of Experimental Medicine* 145: 726-42.

☐ One of the first uses of proteins with known mutations leading to alterations in structure as antigens. It was possible to determine the differences between immunodominat groups required for antibody production as opposed to cellular responses.

1978.
Stingl, G.; Katz, S. I.; Shevach, E. M.; Rosenthal, A. S.; and Green, I. Analogous Functions of Macrophages and Langerhans Cells in the Initiation of the Immune Response. *Journal of Investigatory Dermatology* 71: 59-64.

☐ One of the first descriptions of the importance of the skin Langerhans cells in antigens.

1978.
Platshon, L., and Kaliner, M. The Effect of the Immunologic Release of Histamine Upon Human Lung Cyclic Nucleotide Levels and Prostaglandin Generation. *Journal of Clinical Investigation* 62: 1113-21.

☐ This article demonstrated that mediator release from human lung mast cells had a profound effect on a number of cell types within the human lung and demonstrated that prostaglandin formation was a secondary event in response to human mast cell-derived mediators including histamine. This is the first analysis of such a response in human lung and led to a complete analysis of the role of histamine acting through H1 and H2 receptors as well as the identification of prostaglandin generating factor of anaphylaxis.

1979.
Frank, Michael M.; Hamburger, Max I.; Lawley, Thomas J.; Kimberly, Robert P.; and Plotz, Paul H. Defective Reticuloendothelial System Fc-Receptor Function in Systemic *Lupus Erythematosus*. *New England Journal of Medicine* 300: 518-23.

☐ Autologous erythrocytes with either IgG or complement fragments deposited on them *in vitro* were infused into the erythrocyte donor. These cells are removed from the circulation by the action of very specific receptors for these molecules on the cells of the reticuloendothelial system. The functional status of these cells could be determined. Patients with circulating immune complexes that were deposited in tissues were shown to have defect *in vivo* function of the IgG Fc receptor.

1979.
Henderson, W. R.; Shelhamer, J. H.; Reingold, D. B.; Smith, L. J.; Evans, R.; and Kaliner, M. Alpha Adrenergic Hyperresponsiveness in Asthma—Analysis of Vascular and Puillary Responses. *New England Journal of Medicine* 300: 642-47.

☐ This is the first demonstration of alpha adrenergic hyperreactivity in asthmatic subjects and laid the groundwork for a number of studies that have subsequently gone on to analyze peripheral responses in asthmatic subjects

1979.
Rosenwasser, L. J.; Dinarello, C. A.; and Rosenthal, A. S. Adherent Cell Function in Murine T Lymphocyte Antigen Recognition. IV. Enhancement of Murine T Cell Antigen Recognition by Human Leukocytic Pyrogen. *Journal of Experimental Medicine* 150: 709-14.

☐ First demonstration that endogenous pyrogens was the same substance as LAF (IL-1).

1981.
Lawley, Thomas J.; Hall, Robert P.; Fauci, Anthony S.; Katz, Stephen I.; Hamburger, Max I.; and Frank, Michael M. Defective Fc Receptor Functions Associated with the HLA—B8/DRw3 Haplotype. *New England Journal of Medicine* 304: 185-92.

☐ HLA—B8/DRw3 is associated with the development of a wide variety of autoimmune diseases. This appear demonstrates that these individuals have a defect in their ability to clear immune complexes from their circulation before they become ill.

1982.
Joiner, Keith A.; Hammer, Carl H.; Brown, E. J.; and Frank, Michael M. Studies on the Mechanism of Bacterial Resistance to Complement-Mediated Killing. II. C8 and C9 Release C5b67 from the Surface of *Salmonella minnesota* S218 Because the Terminal Complex Does Not Insert into the Bacterial Outer Membrane. *Journal of Experimental Medicine* 155: 809-919.

☐ The first detailed analysis of the mechanisms that allow a serum resistant gram negative bacterium to resist complement attack. The paper opened the door to a series of studies that demonstrated that microorganisms have evolved a series of strategies to protect themselves from the host defenses as mediated by antibody and complement.

1983.
Gallin, J. I.; Buescher, E. S.; Seligmann, B. E.; Gaither, T.; Nath, J.; and Katz, P. Recent Advances in Chronic Granulomatous Disease. Conference of the NIH Combined Clinical Staffs. *Annals of Internal Medicine* 99: 657-74.

☐ First review pointing out that chronic granulomatous disease of childhood is a group of disorders of phagocyte oxidative metabolism.

1983.
Joiner, Keith A.; Goldman, Robert C.; Hammer, Carl H.; Lieve, Loretta; and Frank, Michael M. Studies on the Mechanism of Bacterial Resistance to Complement-Mediated Killing. *Journal of Immunology* 131: 2563-69.

☐ One of a series of papers that demonstrated that antibody directs complement attack. The same number of complement molecules may deposit on a bacterial surface in the presence and absence of antibody. Those deposited in the presence and absence of antibody. Those deposited in the presence of bactericidal antibody will kill the organism. Those deposited in its absence will not.

1984.
Gallin, J. I. Neutrophil Specific Granules: A Fuse that Ignites the Inflammatory Response. *Clinical Research* 32: 320-28.

☐ Summary of work done at NIH demonstrating the storage of neutrophil plasma membrane receptors and mediators of inflammation within neutrophil specific granules. Text of address to American Federation of Clinical Research upon receipt of Young Investigator Award.

1984.
Lawley, Thomas J.; Bierlory, Leonard; Gascon, Pedro; Yancey, Kim B.; Young, Neal S.; and Michael, Frank M. A Prospective Clinical and Immunologic Analysis of Patients with Serum Sickness. *New England Journal of Medicine* 311: 1407-13.

☐ The first prospective study of serum sickness in man. A new clinical sign was demonstrated and the induction of arthritis in man was demonstrated.

Laboratory of Immunogenetics (LIG)

Established: 1977



Chief: Thomas J. Kindt, Ph.D.
1977-

History:
The Laboratory of Immunogenetics was established to provide an intramural focus for research in immunogenetics. The laboratory investigates the multigene families that are involved in control of immune function. A wide range of techniques at the molecular, serologic, and functional level are used to carry out their studies.

The Laboratory of Immunogenetics originated in July 1977 with three investigators from Rockefeller University: Drs. John A. Sogn, John E. Coligan, and Thomas J. Kindt. The major projects then underway in the laboratory involved the structural determination by radiochemical methods of the murine H-2Kb protein; the molecular basis for unpredicted allotypes in laboratory rabbits; and studies of MHC antigens by immunochemical techniques. Programs of the laboratory have been characterized by a molecular approach to questions in immunology and have evolved with the development of new technologies and concepts. An offshoot of the protein sequence studies came with the recognition that peptide antibodies could be used to link molecular biological information to protein expression. Recently, the protein and peptide technology have been incorporated into a service branch headed by Dr. Coligan, who will provide service in those areas for the entire intramural program. Dr. Sogn has taken an administrative position in the National Cancer Institute leaving only Dr. Kindt from the original group.

Presently, other senior investigators in LIG are Dr. Eric Long, who studies the molecular basis of HLA class II antigen function; Dr. Ed Max, who studies regulator control of genes in B cells; and Dr. Mary Ann Robinson, who carries genetic studies of HLA and T cell receptor genes.

1981.
Coligan, John E.; Kindt, Thomas J.; Uehara, H.; Martinko, J. M.; and Nathenson; S. G. Primary Structure of a Murine Transplantation Antigen. *Nature* 291: 35-39.

☐ This paper was listed in the 100 most cited papers of 1982 because it was the first to report the complete primary structure of a major histocompatibility complex antigen.

1981.
Gates, Frederick T., III; Coligan, John E.; and Kindt, Thomas J. Complete Amino Acid Sequence of Murine β_2-microglobulin: Structural Evidence for Strain-related Polymorphism. *Proceedings of the National Academy of Sciences, USA 78: 554-58.*

☐ Complete sequence data were obtained by radiochemical techniques for β_2-microglobulin from two different mouse strains showing that a single amino acid interchange constituted the allelic difference between them.

1981.
Yarmush, Martin L.; Sogn, John A.; Kern, Paul D.; and Kindt, Thomas J. Role of Immune Recognition in Latent Allotype Induction and Clearance. Evidence for an Allotypic Network. *Journal of Experimental Medicine* 153: 196-206.

☐ It was shown that injection of anti-allotype antibodies could induce expression of immunoglobulin allotypes not expected from the genotype thus extending the network hypothesis to include allotypes as well as idiotypes.

1983.
Emorine, Laurent; Dreher, Kevin; Kindt, Thomas J.; and Max, Edward E. Rabbit Immunoglobulin K Genes: Structure of a Germline b4 Allotype J-C Locus and Evidence for Several b4-Related Sequences in the Rabbit Genome. *Proceedings of the National Academy of Sciences, USA* 80: 5709-13.

☐ Cloning and sequence analysis of a rabbit K immunoglobulin gene revealed a cluster of five J region segments, a locus unique in the immunoglobulin gene family in having only a single functional J segment within the cluster.

1983.
Emorine, Laurent; Kuehl, Michael; Weir, Lawrence; Leder, P.; and Max, Edward E. A Conserved Sequence in the Immunoglobulin J_k-C_k Intron: Possible Enhancer Element. *Nature* 304: 447-49.

☐ A three species nucleotide sequence comparison of the DNA in the intron lying between the J and C regions of the immunoglobulin kappa gene defined an isolated $\geq$0.3 kb conserved region that has subsequently been shown to be a critical role in regulating expression of the gene.

1984.
Kindt, Thomas J., and Capra, J. D. *The Antibody Enigma*. New York: Plenum Press 257 p.

☐ This book traces the scientific progress leading to an understanding of how antibody genes are able to encode an almost unlimited potential to respond to foreign antigens.

1984.
Robinson, Mary A.; Long, Eric O.; Johnson, A. H.; Hartzman, R. J.; Mach, B.; and Kindt, Thomas J. Recombination Within the HLA-D Region. Correlation of Molecular Genotyping with Functional Data. *Journal of Experimental Medicine* 160: 222-38.

☐ Molecular analyses of MHC genes in families with recombinant HLA haplotypes located crossover points and found new recombinants that could be confirmed by cellular analyses.

1984.
Tykocinski, Mark L., and Max, Edward E. CG Dinucleotide Clusters in MHC Genes and in 5′ Demethylated Genes. *Nucleic Acids Research* 12: 4385-96.

☐ It was observed that the dinucleotide CpG, which is found at a much lower frequency in mammalian DNA than statistically expected on the basis of total C and G content, occurs with a sharply higher frequency in the 5′ regions of certain genes, particularly certain MHC genes.

1985.
Max, Edward E., and Korsmeyer, S. J. Human J Chain Gene. Structure and Expression in B Lymphoid Cells. *Journal of Experimental Medicine* 161: 832-49.

☐ This analysis of a cloned human J chain gene established the exon-intron structure of the genomic gene and the correct amino acid sequence of the protein, revealed the existence of an unexpected related sequence in the human genome, and provided data on the expression of J chain mRNA at different stages in the development of B lymphocytes.

1985.
Robinson, Mary A., and Kindt, Thomas J. Segregation of Polymorphic T Cell Receptor Genes in Human Families. *Proceedings of the National Academy of Sciences, USA* 82: 3804-8.

☐ This paper documents the first demonstration of genetic variation in genes encoding the T cell antigen receptor in man.

1985.
Tonnelle, Cecille; DeMars; R.; and Long, Eric O. Doa: A New β Chain Gene in HLA-D with a Distinct Regulation of Expression. *EMBO Journal* 4: 2839-47.

☐ This paper describes the discovery of a new HLA gene and documents its highly specific pattern of expression that indicated that the encoded antigen may be a marker for mature B cells.

1986.
Lew, Andrew M.; Margulies, D. H.; Maloy, Walter L.; Lillehoj, Erik P.; McCluskey, James; and Coligan, John E. Alternative Protein Products with Different Carboxyl Termini from a Single Class I Gene, H-2Kb. *Proceedings of the National Academy of Sciences, USA* 83: 6084-88.

☐ First protein evidence that individual major histocompatibility complex class I genes can be encoded for more than one gene product suggesting that novel mechanisms may increase the class I repertoire.

1986.
Lew, Andrew M.; Pardholl, Drew M.; Maloy, Walter L.; Fowlkes, B. J.; Kruisbeek, Ada; Cheng, S.-F.; Germain, Ronald N.; Bluestone, Jeffery A.; Schwartz, Ronald H.; Coligan, John E. Characterization of T Cell Receptor Gamma Chain Expression in a Subset in Murine Thymocytes. *Science* 234: 1401-5.

☐ First description in the thymus of a second T lymphocyte antigen receptor (termed gamma-delta) which has subsequently been shown to be the first receptor expressed during ontogenesis.

1986.
Marche, Patrice N., and Kindt, Thomas J. Two Distinct T Cell Receptor-Chain Transcripts in a Rabbit T Cell Line: Implications for Allelic Exclusion in T Cells. *Proceedings of the National Academy of Sciences, USA* 83: 2190-94.

☐ This is the first description of T cell receptor alpha genes in the rabbit and sequence analysis showed two full-length transcripts from the same cell indicating that RNA production is not sufficient to stop the synthesis of antigen receptors subject to allelic exclusion.

Laboratory of Immunology (LI)

Established: 1957

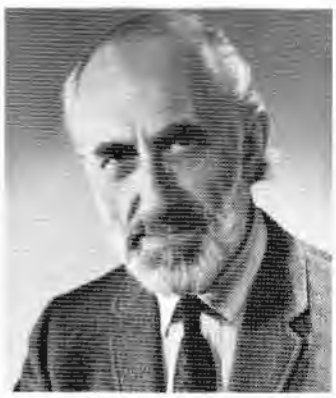


Chief: William E. Paul, M.D.
1971-

Prior:



Baruj Benacerraf, M.D.
1968-70



Maurice Landy, Ph.D.
1962-67



Jules Freund, M.D.
1957-60

History:
The establishment of this laboratory was the result of an awareness of the extent of allergic disorders and the increasingly important role of the discipline of immunology in coping with key issues in experimental biology and medicine. Culminating several NIH and Institute studies and proposals during the prior year, the decision to found a separate Laboratory of Immunology was made during the latter part of 1956. Dr. Freund was appointed the first Chief on January 2, 1957. This new laboratory took shape during the early part of 1957 with reassignments of personnel from other laboratories of NIAID, primarily from LTD, which had previously carried on basic and clinical research in Immunology.

As it has evolved, the laboratory has expanded to study various aspects of cellular immunology, molecular genetics, immunogenetics, and immunochemistry, particulary as they bear on a fuller understanding of the fundamental mechanisms and biological significance of the immune response.

1970.
Ellman, L.; Green, I.; Martin, W. J.; and Benacerraf, B. Linkage Between the Poly-L-lysine Gene and the Locus Controlling the Major Histocompatibility Complex in Strain 2 Guinea Pigs. *Proceedings of the National Academy of Science, USA* 66: 322-28.

☐ This work established that immune response genes in guinea pigs were linked and were part of the major histocompatibility complex, representing a key step in the development of our understanding of the genetic control of immune responses.

1971.
Paul, W. E.; Katz, D. H.; and Benacerraf, B. Augmented Anti-SIII Antibody Responses to an SIII-Protein Conjugate. *Journal of Immunology* 107: 685-88.

☐ The demonstration that anti-polysaccharide responses could be markedly enhanced as a result of T cell sensitization to a protein to which the polysaccharide epitope was linked. This observation formed the basis of current strategies to increase the immunogenicity of polysaccharide vaccines by linking them to proteins and may be of particular value in efforts to immunize infants.

1972.
Lieberman, R.; Paul, W. E.; Humphrey, W. Jr.; and Stimpfling, J. H. H-2 Linked Immune Response (Ir) Genes. Independent Loci for *Ir-IgG* and *Ir-IgA* Genes. *Journal of Experimental Medicine* 136: 1231-40.

☐ This was the first demonstration of the existence of a second genetic locus within the major histocompatibility complex that controlled immune responses to specific antigens. It initiated a long series of studies that led to the mapping of Ir gene control of a large series of proteins.

1972.
Shevach, E. M.; Herberman, R.; Frank, M. M.; and Green, I. Receptors for Complement and Immunoglobulin on Human Leukemic Cells and on Human Lymphoblastoid Cell Lines. *Journal of Clinical Investigation* 51: 1933-38.

☐ This paper represents the initial effort to classify lymphoid tumors based on their surface markers. It initiated a large series of studies that have led to a precise understanding of the origin of the malignant cells and have been of great value in the diagnosis and therapy of leukemias and lymphomas.

1972.
Shevach, E. M.; Paul, W. E.; and Green, I. Histocompatibility Linked Immune Response Gene Function in Guinea Pigs: Specific Inhibition of Antigen-Induced Lymphocyte Proliferation by Alloantisera. *Journal of Experimental Medicine* 136: 1207-21.

☐ This paper demonstrated that class II major histocompatibility complex molecules were specifically recognized by T cells in the course of T cell-macrophage interactions in T cell responses and thus was the key observation in the demonstration that class II molecules were immune response gene products.

1972.
van Boxel, J.; Paul, W. E.; Green, I.; and Terry, W. D. IgD Bearing Human Lymphocytes. *Journal of Immunology* 109: 648-54.

☐ This was the first description of the fact that IgD was basically a cell surface receptor molecule of B cells and thus represented the initial recognition of the function of this class of immunoglobulin.

1973.
Rosenthal, A. S., and Shevach, E. M. Function of Macrophages in Antigen Recognition by Guinea Pig T Lymphocytes. I. Requirement for Histocompatible Macrophages and Lymphocytes. *Journal of Experimental Medicine* 138: 1194-1212.

☐ This paper demonstrated that cellular interactions between specific T cells and macrophages required that the cells be of the same major histocompatibility type. It opened the field of histocompatibility restriction of cellular interactions.

1976.
Shevach, E. M.; Frank, M. M.; and Green, I. Linkage of the Gene Controlling the Synthesis of the Fourth Component of Complement to the Major Histocompatibility Complex of the Guinea Pig. *Immunogenetics* 3: 596-601.

☐ The first demonstration that the C4 gene was part of the major histocompatibility complex. This has had important implications in understanding the genetic organization of the MHC.

1977.
Paul, W. E., and Benacerraf, B. Functional Specificity of Typhus-Dependent Lymphocytes. A Relationship Between the Specificity of T Lymphocytes and Their Function is Proposed. *Science* 195: 1293-1300.

☐ A landmark review in which an influential proposal was made that integrated immunological specificity and function of T lymphocytes.

1977.
Paul, W. E.; Shevach, E. M.; Pickeral, S.; Thomas, D. W.; and Rosenthal, A. S. Independent Populations of Primed F_1 Guinea Pig T Lymphocytes Respond to Antigen-Pulsed Parental Peritoneal Exudate Cells. *Journal of Experimental Medicine* 145: 618-30.

☐ This observation established that T cells restricted by class II MHC molecules were actually co-specific for antigen and class II MHC molecules and represented a key step in establishing that T cells recognized a complex class II and antigenic determinants.

1980.
Schwartz, R. H.; Chen, C.; Paul, W. E. Gene Complementation in the T Lymphocyte Proliferative Response to Poly(Glu^{56}Lys^{35}Phe^{9}). Functional Evidence for a Restriction Element Coded by Both I-A and I-E Subregions *European Journal of Immunology* 10: 708-14.

☐ This paper provided convincing functional evidence that IR gene products and class II MHC restriction elements were identical.

1980.
Sredni, B., and Schwartz, R. H. Alloreactivity of an Antigen-Specific T Cell Clone. *Nature* 287: 855-57.

☐ The demonstration that antigen-specific, MHC-restricted T cells are often specific for allo-MHC determinants, leading to an explanation of high frequency of alloreactive T cells and providing critical information toward understanding of nature of the T cell specificity repertoire.

1980.
Sredni, B.; Tse, H. Y.; and Schwartz, R. H. Direct Cloning and Extended Culture of Antigen-Specific MHC-Restricted, Proliferating T Lymphocytes. *Nature* 287: 855-57.

☐ The first demonstration of one of the two major methods for the preparation of antigen-specific cloned long term T cell lines.

1980.
Stingl, G.; Katz, S. E.; Green, I.; and Shevach, E. M. The Functional Role of Langerhans Cells. *Journal of Investigative Dermatology* 74: 325-28.

☐ The demonstration that epithelial Langerhans cell are critical antigen-presenting cells and that these cells may have important physiologic and pathophysiologic functions in skin-mediated sensitization reactions.

1982.
Howard, M.; Farrar, J.; Hilfiker, M.; Johnson, B.; Takatsu, K,; Hamaoka, T.; and Paul, W. E. Identification of a T Cell-Derived B Cell Growth Factor Distinct From Interleukin-2. *Journal of Experimental Medicine* 155: 914-23.

☐ The initial description of the lymphokine now referred to as interleukin-4/B cell stimulatory factor-1 and the demonstration of its role as a co-stimulant of B cell growth.

1982.
Paul, W. E., and Bona, C. Regulatory Idiotypes and Immune Networks: A Hypothesis. *Immunology Today* 3: 230-34.

☐ The initial proposal of the existence of specialized set of idiotopes with regulatory function and of the special roles of these determinants in the immune network.

1983.
Heber-Katz, E.; Hansburg, D.; and Schwartz, R. H. The Ia Molecule of the Antigen-Presenting Cell Plays a Critical Role in Immune Response Gene Regulation of T Cell Activation. *Journal of Molecular and Cellular Immunology* 1: 3-14.

☐ The demonstration of unambiguous function evidence for the existence of a trimolecular complex involving antigen, class II MHC molecules, and T cell receptors in the T cell activation process.

1984.
Davis, M. M.; Cohen, D. I.; Nielsen, E. A.; Steinmetz, M.; Paul, W. E.; and Hood, L. Cell-Type Specific cDNA Probes and the Murine I Region: The Localization and Orientation of $A\alpha^d$. *Proceedings of the National Academy of Sciences,* USA 81: 2194-98.

☐ The introduction of the technique of subtractive hybridization to identify tissue-specific genes and the use of that technique to isolate the gene for the class II $A\alpha$ chain.

1984.
Germain, R; Norcross, M.; and Margulies, D. Functional Expression of a Transfected Murine Class II MHC Gene. *Nature* 306: 190-94.

☐ The direct demonstration by DNA-mediated gene transfer techniques that class II MHC molecules are the co-recognition elemeuts in T cell recognition.

1984.
Hedrick, S. M.; Cohen, D. I.; Nielsen, E. A.; and Davis, M. M. Isolation of cDNA Clones Encoding T Cell-Specific Membrane Associated Proteins. *Nature* 308: 149-53.

☐ The first molecular cloning of the gene for the β chain of the T cell receptor for antigen. This paper opened the field of study of the molecular biology of T cell receptors and of the molecular and cellular mechanisms for the generation of the T cell repertoire.

1985.
Germain, R. N.; Bentley, D. M.; and Quill, H. Influence of Allelic Polymorphism on the Assembly and Surface Expression of Class II MHC (Ia) Molecules. *Cell* 43: 233-42.

☐ The first demonstration that pairing a class II α and β chains displays allelic specificity providing evidence for the specificity of this pairing function.

1985.
Lamoyi, E., and Mage, R. G. The Lack of K1b9 Light Chains in Basilea Rabbits is Probably Due to a Mutation in an Acceptor Site for mRNA Splicing. *Journal of Experimental Medicine* 162: 1149-60.

☐ A demonstration of the molecular basis of the failure to produce an immunoglobulin light chain isotype. Has importance in understanding thecular mechanisms that lead to other instances of immunodeficiency.

1985.
Ohara, J., and Paul, W. E. B Cell Stimulatory Factor (BSF)-1: Production of a Monoclonal Antibody and Molecular Characterization. *Nature* 315: 333-36.

☐ The purification of interleukin-4/B cell stimulatory factor-1 and production of a monoclonal antibody. This paper represents a key step in the molecular and functional characterization of this important lymphokine.

1985.
Samelson, L. E.; Lindsten, T.; Fowlkes, B. J.; van den Elsen, P.; Terhorst, C.; Davis, M. M. The Expression of Genes of the T Cell Antigen Receptor Complex in Precursor Thymocytes. *Nature* 315: 765-68.

☐ The initial description of the molecular events associated with T cell maturation in the thymus as they relate to the expression of the T cell receptor and associated molecules.

1985.
Vitetta, E. S.; Ohara, J.; Myers, C.; Layton, J.; Krammer, P. H.; and Paul, W. E. Serologic, Biochemical and Functional Identity of B Cell Stimulatory Factor-1 and B Cell Differentiation Factor for IgG1. *Journal of Experimental Medicine* 162: 1726-31.

☐ This paper establishes that interleukin-4/B cell stimulatory factor-1.

1986.
Coffman, R. L.; Ohara, J.; Bond, M. W.; Carty, J.; Zlotnik, A.; and Paul, W. E. B Cell Stimulatory Factor-1 Enhances the IgE Response of Lipopolysaccharide-Activated T Cells. *Journal of Immunology* 136: 4538-41.

☐ Interleukin-4/B cell stimulatory factor-1 is the IgE switch factor.

1986.
Finkelman, F. D.; Katona, I.; Urban, J.; Snapper, C.; Ohara, J.; and Paul, W. E. Suppression of *In Vivo* Suppression of *In Vivo* Polyclonal IgE Responses by Monoclonal Antibody to the Lymphokine BSF-1. *Proceedings of the National Academy of Sciences, USA* 83: 9675-78.

☐ The demonstration that interleukin 4/B cell stimulatory factor-1 is the principle in vivo regulator if IgE synthesis in a model of parasite infection. A critical observation in the design of strategies to control IgE synthesis in parasite infections in allergic responses.

1986.
Kroczek, R. A.; Gunther, K. C.; Germain, R. N.; and Shevach, E. M. Thy 1 Functions as a Signal Transduction Molecule in T-Lymphocytes and Transfected B Lymphocytes. *Nature* 181-84.

☐ The direct demonstration by DNA-mediated gene transfer techniques that Thy 1 is capable of transducing biochemical growth-inducing signals in lymphocytes.

1986.
Lechler, R. I.; Ronchese, F.; Braunstein, N. S.; and Germain, R. N. I-A Restriction Antigen-Recognition: Analysis of the Roles of Aα and Aβ using DNA-Mediated Gene Transfer. *Journal of Experimental Medicine* 163: 678-96.

☐ The analysis of structure function relationships in the role of class II MHC molecules in antigen-recognition by T cell receptors.

1986.
Margulies, D. H.; Ramsey, A. L.; Boyd, L. F.; and McCluskey, J. Genetic Engineering of an H-2D^{d}/Q10^{b} Chimeric Histocompatibility Antigen: Purification of Soluble Protein from Transformant Cell Supernatants. *Proceedings of the National Academy of Sciences, USA* 83: 5252-56.

☐ The development of a general technique to produce large quantities of secreted, soluble forms of class I MHC molecules. This approach should be of great value in structural studies of class I molecules and in the study of T cell receptor-binding to class I molecules.

1986.
Mosmann, T. R.; Bond, M. W.; Coffman, R. L.; Ohara, J.; and Paul, W. E. T Cell and Mast Cell Lines Respond to B Cell Stimulatory Factor-1. *Proceedings of the National Academy of Sciences,* USA 83: 5654-58.

☐ The initial recognition that interleukin-4/B cell stimulatory factor-1 has activity on a wide range of hematopoietic lineage cells and is not limited to B cells in its action.

1986.
Saito, T.; Weiss, A.; Miller, J.; Norcross, M. A.; and Germain, R. N. Specific Antigen-Ia Activation of Transfected Human T Cells Expressing Murine Ti αβ-Human T3 Complexes. *Nature* 325: 125-29.

☐ Through the use of DNA-mediated transfer of genes for the T cell receptor α and β chains, it was established that a single receptor is responsible for genetic restriction and for antigen recognition.

1987.
Paul, W. E. Between Two Centuries. Specificity and Regulation in the Immune Response. *Journal of Immunology* 139: 1-6.

☐ American Association of Immunologist presidential address describing the importance of the development of a modern, science of immunoregulation and of understanding the *in vivo* physiology of immune response.

1987.
Snapper, C. M., and Paul, W. E. Interferon Gamma and B Cell Stimulatory Factor-1 Reciprocally Regulate Ig Isotype Production. *Science* 236: 944-47.

☐ The demonstration that interferon gamma controls switching to IgG2a and the proposal of a hypothesis for the T cell mediated control of Ig isotypes based on their relative protective value in viral and parasitic infections.

1987.
Stingl, G.; Koning, F.; Yamada, H.; Yokoyama, W. M.; Tschachler, E.; Bluestone, J. A.; Steiner, G.; Samelson, L. E.; Lew, A. M.; Coligan, J. E.; and Shevach, E. M. Thy-1 + Dendritic Epithelial Cells Express T3 Antigen and the T Cell Receptor Gamma Chain. *Proceedings of the National Academy of Sciences,* USA 84: 2430-34.

A demonstration that a population of epidermal T cells have a heightened expression of a subset of T cells that use the T cell receptor γ chain, probably together with the δ chain, in the formation of their receptor. This work provides an approach to the study of the function of γδ-cells and to the isolation of the T cell receptor δ chain for chemical studies and for cloning.

Laboratory of Immunopathology (LIP)

Established: 1985



Chief: Herbert C. Morse, III, M.D.
1985-

History:
The Laboratory of Immunopathology conducts research on the biology of virus infections in relation to the development and function of the hematopoeitic system. It was established in May 1985 from the Laboratory of Viral Diseases.

1985.
Davidson, Wendy F.; Holmes, Kevin L.; Roths, John B.; and Morse, Herbert C., III. Immunologic Abnormalities of Mice Bearing the *gld* Mutation Suggest a Common Pathway for Murine Nonmalignant Lymphoproliferative Disorders with Autoimmunity. *Proceedings of the National Academy of Sciences,* USA 821: 1219-23.

☐ Studies of mice mutant for the autosomal recessive mutations *lpr* and *gld* indicated that these defects may alter different enzymes in a common metabolic pathway associated with the development of autoimmune disease.

1985.
Lewis, Andrew M., Jr., and Cook, James L. A New Role for DNA Virus Early Proteins in Viral Carcinogenesis. *Science* 227: 15-20.

☐ This study contributes to the continuing pursuit of how tumor virus genes alter normal cells to produce neoplastic cells that may or may not be tumorigenic by describing previously unrecognized functions of DNA tumor virus early genes—namely their ability to induce in transformed rodent cells specific susceptibilities or resistances to lysis by natural killer cells or activated macrophages—that correlate with their tumor inducing capacities in a quantitative model of transformed cell tumorigenicity.

Laboratory of Immunoregulation (LIR)

Established: 1980



Chief: Anthony S. Fauci, M.D.
1980-

History:
This laboratory was established to apply new knowledge in immunology to the clinical diagnosis and treatment of patients with immunological disorders.

1974.
Fauci, Anthony S., and Dale, David C. The Effect of *In Vivo* Hydrocortisone on Subpopulations of Human Lymphocytes. *Journal of Clinical Investigation* 53: 240-46.

☐ This paper demonstrated that corticosteroid administration to man results in a profound, but transient selective depletion of T lymphocytes from the circulation. This was the first observation that single doses of corticosteroids could have a significant effect on the circulatory capabilities of human lymphocytes and caused depletion not by lympholysis, but by altering circulatory kinetics.

1976.
Fauci, Anthony S., and Pratt, Karen R. Polyclonal Activation of Bone-Marrow-Derived Lymphocytes From Human Peripheral Blood Measured by a Direct Plaque-Forming Cell Assay. *Proceedings of the National Academy of Sciences, USA* 73: 3676-79.

☐ This study represents the development of the first assay in the human system for the induction and measurement of antibody production by human lymphocytes at the single cell level.

1976.
Fauci, A. S.; Dale, D. C.; aud Balow, J. E. Glucosorticosteroid Therapy: Mechanism of Action and Clinical Considerations. *Annals of Internal Medicine* 84: 305-15.

☐ This paper is considered the classic paper in delineation of the mechanisms of action of corticosteroids in humans. It received the Citation Classic designation from *Current Contents.*

1978.
Fauci, A. S.; Haynes, B. F.; and Katz, P. The Spectrum of Vasculitis: Clinical Immunologic and Therapeutic Consideration. *Annals of Internal Medicine* 89: 660-76.

☐ This paper established the classification scheme for the vasculitic syndromes which is currently used worldwide and which has become the classic in the field.

1979.
Fauci, A. S.; Katz, P; Haynes, B. F.; and Wolff, S. M. Cyclophosphamide Therapy of Severe Systemic Necrotizing Vasculitis. *New England Journal of Medicine* 301: 235-38.

☐ This was the first paper to demonstrate the efficacy of cyclophosphamide in inducing long-term remissions in systemic vasculitis.

1979.
Hayes, Barton F.; Eisenbarth, George S.; and Fauci, Anthony S. Human Lymphocyte Antigens: Productions of a Monoclonal Antibody that Defends Functional Thymus-Derived Lymphocyte Subsets. *Proceedings of the National Academy of Sciences, USA* 76: 5929-33.

This paper reported the development of a monoclonal antibody against a distinct subset of human T cells and helped ultimately to map intrathymic development of T cell repertoire.

1981.
Lane, Clifford H.; Volkman, David J.; Whalen, Gail; and Fauci, Anthony S. *In Vitro* Antigen-Induced, Antigen-Specific Antibody Production in Man. Specific and Polyclonal Components, Kinetics, and Cellular Requirements. *Journal of Experimental Medicine* 154: 1043-57.

☐ This study was the first to demonstrate antigen-induced, antigen-specific antibody production in human peripheral blood. It has since laid the framework for the study of the specificity of the human immune response to soluble antigens.

1982.
Lane, Clifford H.; Shelmaner, James H.; Mostowski, Howard S.; and Fauci, Anthony S. Human Monoclonal Anti-Keyhole Limpet Hemocyanin Antibody-Secreting Hybridoma Produced from Peripheral Blood B Lymphocytes of a Keyhold Limpet Hemocyanin-Immune Individual. *Journal of Experimental Medicine* 155: 333-38.

☐ This paper describes the first hybridoma developed from human peripheral blood which secretes monoclonal antibody to a soluble antigen against which the subject was immunized. It proved the feasibility of developing human monoclonal antibodies against predetermined antigenic specificities.

1983.
Fauci, Anthony S.; Haynes Barton, F.; Katz, Paul; and Wolff, Sheldon M. Wegener's Granulomatosis: Prospective Clinical and Therapeutic Experience with 85 Patients for 21 Years. *Annals of Internal Medicine* 98: 76-85.

☐ This paper is the classic work in the field of therapy for Wegener's granulomatosis and the vasculitic syndromes. It describes the cumulative experience over 2 decades with the use of cyclophosphamide therapy in the cure of this previously fatal disease. It is one of several papers over the years reporting on these pioneering studies.

1985.
Lane, Clifford H.; Depper, Joel, M.; Greene, Warner C.; Whalen, Gail; Waldmann, Thomas A.; and Fauci, Anthony S. Qualitative Analysis of Immune Function in Patients with the Acquired Immunodeficiency Syndrome. Evidence for a Selective Defect in Soluble Antigen Recognition. *New England Journal of Medicine* 313: 79-84.

☐ This paper describes the selectivity of the immunologic defect in AIDS. It establishes the basis for the dissection of the immunopathogenic mechanisms related to infection with the human immunodeficiency virus.

1985.
Ambrus, Julian L., Jr.; Jurgensen, Cynthia H.; Brown, Eric J.; and Fauci, Anthony S. Purification to Homogenicity of a High Molecular Weight Human B Cell Growth Factor; Demonstration of Specific Binding to Activated B Cells; and Development of a Monoclonal Antibody to the Factor. *Journal of Experimental Medicine* 162: 1319-35.

☐ This paper describes the purification of a high molecular weight human B cell growth factor and the demonstration of its specific binding to activated B cells. It is one of a series of papers from the Laboratory of Immunoregulation which have delineated the mechanisms of regulation of the human B cell cycle.

1986.
Folks, T.; Powell, D. M.; Lightfoote, M. M.; Benn, S.; Martin, M. A.; and Fauci, A. S. Induction of HTLV-III/LAV Expression from a Nonvirus-Producing T Cell Line: Implications for Latent Infection in Man. *Science* 231: 600-02

☐ This study established a model system for the study of latency of infection with the AIDS virus and the mechanisms of conversion of a latent infection to a productive infection. It has assumed major importance in our understanding of the immunopathogenesis of infection with the human immunodeficiency virus (HIV).

Laboratory of Infectious Diseases (LID)

Established: 1948 with the formation of NMI. Existed in other forms since August 1887.



Chief: Robert M. Chanock, M.D.
1968-

Prior:



Robert J. Huebner, M.D.
1956-67



Dorland J. Davis, M.D.
1954-56



Karl Habel, M.D.
1948-54



Charles Armstrong, M.D.
~~1942~~-48
1941-48
VAH –
4-4-05

History:
This laboratory is the most direct descendant of the original Marine Hospital Service research laboratory established in August 1887 at the Marine Hospital on Staten Island, New York. At that time its function was to assist the Service in diagnosing infectious diseases among passengers on incoming ships. Detached from the hospital and moved to the Butler Building in Washington, D.C. in 1891, this "Laboratory of Hygiene" became known as the Hygienic Laboratory. Authorized in 1901 by Congress to investigate "infectious and contagious diseases and matters pertaining to the public health," the research of the Hygienic Laboratory focused primarily on bacteriology and pathology, the two major fields of nineteenth century medical interest. In 1902 a reorganization of the Service divided the Laboratory into four divisions; infectious disease research was located in the Division of Pathology and Bacteriology.

On February 1, 1937, the Division was renamed the Division of Infectious Diseases, becoming one of the eight Divisions and one Office (of Cooperative Studies) defined in the National Institute of Health reorganization of that date. At this time, the Division was assigned administrative jurisdiction over the Rocky Mountain Laboratory, and the Division incidentally carried on the heart and dental research of the Public Health Service.

In the reorganization of November 1948 that created the (plural) National Institutes of Health, the Division became the Laboratory of Infectious Diseases, one of the four original components of the National Microbiological Institute (NMI), also established at that time. The Rocky Mountain Laboratory was assigned equal administrative status as part of the new NMI, and heart and dental activities were placed in their own newly created Institutes.

1972
Kapikian, A. Z.; Wyatt, Richard G.; Dolin, R.; Thornhill, T.S.; Kalica, Anthony R.; and Chanock, Robert M. Visualization by Immune Electron Microscopy of a 2.7 nm Particle Associated with Acute Infectious Nonbacterial Gastroenteritis. *Journal of Virology* 10: 1075-81.

☐ First recognition of non A, non B hepatitis which now constitutes the major form of post-transfusion hepatitis as well as a common form of sporadic viral hepatitis.

1975.
Purcell, Robert H., and Gerin, J. L. Hepatitis B Subunit Vaccine: A Preliminary Report of Safety and Efficacy Tests in Chimpanzees. *American Journal of Medical Science* 270: 395-99.

☐ First demonstration that purified hepatitis B virus (HBV) surface antigen induces resistance to infection by HBV in the chimpanzee. This observation was critical to the development of the HBV vaccine.

1980.
Lamb, R. A.; Choppin, P. W.; Chanock, Robert M.; and Lai, Ching-J. Mapping of the Overlapping Genes for Polypeptides NS_1 and NS_2 on RNA Segment 8 of the Influenza Virus Genome. *Proceedings of the National Academy of Science, USA 77: 1857-61.*

- *First description of RNA splicing of transcripts from an RNA genome.*

1980.
Wong, Doris C.; Purcell, Robert H.; Sreenivasan, M. A.; Prasad, S. R.; and Pavri, K. M. Epidemic and Endemic Hepatitis in India: Evidence for a Non-A Non-B Hepatitis Virus Etiology. *Lancet* 2: 876-79.

- First evidence for the existence of a second form of non A, non B hepatitis virus that is responsible for large common source (usually water borne) epidemics of hepatitis.

1980.
Rizzetto, M.; Canese, M. G.; Gerin, J. L.; London, William T.; Sly, L. D.; and Purcell, Robert H. Transmisson of the Hepatitis B Virus-Associated Delta Antigen to Chimpanzees. *Journal of Infectious Diseases* 141: 590-602.

- Demonstration that the delta antigen was a transmissible agent which required simultaneous infection by hepatitis B virus for its replication. Thus, delta was shown to be a defective, transmissible virus which required for its replication functions provided by its helper virus, hepatitis B virus. Subsequent studies established an important role for delta virus in the etiology of unusually severe, often fatal viral hepatitis.

1982.
Murphy, Brian R.; Sly, D. L.; Tierney, Eveline L.; Hosier, N. T.; Massicot, J. G.; London, William T.; Chanock, Robert M.; Webster, R. G.; and Hinshaw, V. S. Reassortant Virus Derived from Avian and Human Influenza A Viruses is Attenuated and Immunogenic in Monkeys. *Science* 218: 1330-32.

- Substitution of genes coding for the internal viral and non-structural proteins of an avian influenza A virus for the corresponding genes of a human influenza A virus by gene reassortment attenuates human influenza A virus.

1986.
Kapikan, A. Z.; Flores, Jorge; Hoshino, Yatsutaka; Glass, Roger I.; Midthun, Karen; Gorziglia, Mario; and Chanock, Robert M. Rotavirus: The Major Etiologic Agent of Severe Infantile Diarrhea May be Controllable by a "Jennerian" Approach to Vaccination. *Journal of Infectious Diseases* 153: 815-22.

- Description of "Jennerian" approach to prevention of severe rotavirus diarrhea of infancy and early childhood employing a rotavirus of simian origin which is attenuated but immunogenic in humans.

1986.
Olmstead, Robert A.; Elango, Narayansamu; Prince, Gregory A.; Murphy, Brian R.; Johnson, Philip R.; Moss, Bernard; Chanock, Robert M.; and Collins, P. L. Expression of the F Glycoprotein of Respiratory Syncytial Virus by a Recombinant Vaccinia Virus: Comparison of the Individual Contributions of the F and G Glycoproteins to Host Immunity. *Proceedings of the National Academy of Sciences, USA* 83: 7462-66.

- Construction of vaccinia virus-respiratory syncytial virus (RSV) glycoprotein gene recombinants which express authentic RSV F or G glycoproteins and which induce significant resistance to RSV in the lungs of immunized cotton rats. First description of a successful experimental strategy for immunoprophylaxis of RSV which may have application to the control of this virus which is the most important respiratory tract viral pathogen of infancy and early childhood.

Laboratory of Microbial Immunity (LMI)

Established: 1969



Chief: Richard Asofsky, M.D.
1972-

Prior: (Laboratory of Germfree Animal Research until 1969)



John E. Tobie, Ph.D.
1964-72



Walter L. Newton, Ph.D.
1959-1964

History:
The Laboratory of Microbial Immunity was formally established in 1969. Its investigations are concerned with the elucidation of basic mechanism involved in both the cellular and humoral responses to microbial and tissue antigens. This laboratory developed from the earlier Laboratory of Germfree Animal Research, (LGAR) which was established in 1959.

1970.
Baker, Philip J.; Stashak, Philip W.; Amsbaugh, D. F.; Prescott, B.; and Barth, R. F. Evidence for the Existence of Two Functionally Distinct Types of Cells which Regulate the Antibody Response to Type 111 Pneumococcal Polysaccharide. *Journal of Immunology* 105: 1581-83.

☐ This was one of the earliest papers to show the existence of "suppressor" T lymphocytes. It was also the first to suggest that "T-independent" responses are regulated by T lymphocytes which amplify or suppress the response.

1971.
Pierce, C. W.; Solliday, S. M.; and Asofsky, Richard. Immune Responses *In Vitro*. IV. Suppression of Primary γM, γG, and γA Plaque-Forming Cell Response in Mouse Spleen Cell Cultures by Class-Specific Antibody to Mouse Immunoglobulins. *Journal of Experimental Medicine* 135: 675-97.

☐ Paper showed that anti-IgM blocked antibody responses of all isotypes *in vitro*, and that the block was specific for B cells, indicating that IgM was the major (or only) antigen-specific receptor on B lymphocytes (see Lawton reference).

1972.
Cantor, H., and Asofsky, Richard. Synergy Among Lymphoid Cells Mediating the Graft-*Versus*-Host Response. III. Evidence for Interaction Between Two Types of Thymus-Derived Cells. *Journal of Experimental Medicine* 135: 764-79.

1972.
Tigelaar, R. E., and Asofsky, Richard. Synergy Among Lymphoid Cells Mediating the Graft-*Versus*-Host Response. IV. Synergy in the GVH Reaction Quantitated by a Mortality Assay in Sublethally Irradiated Recipients. *Journal of Experimental Medicine* 135: 1059-70.

☐ These two papers provided important evidence for functional heterogeneity among T lymphocytes, and were the first to show that some T cells would amplify the activities of others.

1972.
Lawton, A. R. III; Asofsky, Richard; Hylton, M. B.; and Cooper, M. D. Suppression of Immunoglobulin Class Synthesis in Mice. 1. Effects of Treatment with Antibody to P-Chain. *Journal of Experimental Medicine* 135: 277-97.

☐ Purified anti-IgM given to mice from birth suppressed completely the development of B lymphocytes and blocked development of antibody responses of all isotypes. The work indicated that all B lymphocytes pass through a stage in which membrane-bound IgM was expressed. Animals treated in this way also provide a useful model of B-lymphocyte deficiency.

1972.
Amsbaugh, Diane F.; Hansen, C. T.; Prescott, B.; Stashak, Philip W.; Barthold, D. R.; and Baker, Philip J. Genetic Control of the Antibody Response to Type III Pneumococcal Polysaccharide in Mice. Evidence that an X-Linked Gene Plays a Decisive Role in Determining Responsiveness. *Journal of Experimental Medicine* 136: 931-49.

☐ The first description of an X-linked immunodeficiency in strain CBA/N mice, now a standard model for studying such deficiencies.

1979.
Pasanen, V. J.; Asofsky, Richard; and Baker, Philip J. Synthesis of Two Classes of Antibody, γM, and γG, or γG and γA by Identical Cells. Amplification of the Antibody Response to Pneumococcal Polysaccharide Type 111. *Journal of Experimental Medicine* 149: 1227-37.

☐ Demonstration of large numbers cells secreting two isotypes of antibody to the same antigen simultaneously ("double-secretors"), providing, direct evidence for an isotype switch in individual cells during development of an antibody response.

1984.
Fowlkes, B. J. Characterization and Differentiation of Thymic Lymphocytes in the Mouse. Ph.D. Thesis. Washington, DC: George Washington University Graduate School of Arts and Sciences.

1985.
Fowlkes, B. J.; Edison, L.; Mathieson, B. J.; and Chused, Thomas M. Early T Lymphocytes: Differentiation *In Vivo* of Adult Intrathymic Precursor Cells. *Journal of Experimental Medicine* 162: 802.

☐ These two works described the isolation of a small subpopulation of thymocytes almost lacking expression of differentiation antigens of T lymphocytes, and showed that all other types of T-cell could be derived from it.

1985.
Baker, Philip J.; Bailey, D. W.; Fauntleroy, M. B.; Stashak, Philip W.; Caldes, G.; and Prescott, B. Genes in Different Chromosomes Influence the Antibody Response to Bacterial Antigens. *Immunogenetics* 22: 269-76.

☐ Paper localized genetic factors controlling antibody responses to polysaccharide antigens to at least 5 different mouse chromosomes, none closely linked to the MHC.

Laboratory of Molecular Microbiology (LMM)

Established: 1981



Chief: Malcolm A. Martin, M.D.
1981-

History:
The Laboratory of Molecular Microbiology was established in 1981 to exploit new methods in recombinant DNA technology and to study other molecular areas thus expanding the Institute's interests in both bacterial and viral pathogenesis and virulence.

1971.
Gelb, L. D.; Kohne, D. E.; and Martin, Malcolm A. 1971. Quantitation of SV-40 Sequences in Green Monkey Mouse and Virus Transformed Cell Genomes. *Journal of Molecular Biology* 57: 129-45.

☐ This paper was the first to quantitate the number of viral genomes integrated into the chromosome of a viral-induced tumor cell.

1971.
Gelb, L. D.; Aaronson, S. A.; and Martin, Malcolm A. Heterogeneity of Murine Leukemia Virus *In Vitro* DNA: Detection of Viral DNA in Mammalian Cells. *Science* 172: 1353-55.

☐ This paper was first to demonstrate that normal mammalian cells (in this case, mouse) contain copies of retroviral sequences.

1972.
Khoury, G., and Martin, Malcolm A. A Comparison of SV40 DNA Transcription *In Vivo* and *In Vitro*. *Nature* 238: 4-6.

☐ This paper was the first to show that *both* strands of a viral DNA can be used to program the synthesis of viral messenger RNA and proteins.

1973.
Khoury, G.; Martin, Malcolm A.; Lee, N. H.; Danna, K. J.; and Nathans, D. A Map of SV40 Transcription Sites Expressed in Productively Infected Cells. *Journal of Molecular Biology* 78: 377-89.

☐ This paper describes the first use of restriction enzymes to prepare transcriptional maps of genes or genomes.

1975.
Howley, P. M.; Mullarkey, M. F.; Takemoto, K. K.; and Martin, Malcolm A. Characterization of Human Papovavirus BK DNA. *Journal of Virology* 15: 173-81.

1975.
Howley, P. M.; Khoury, G.; Byrne, J.; Takemoto, K. K.; and Martin, Malcolm A. Physical Map of the BK Virus Genome. *Journal of Virology* 16: 959-73.

☐ These two papers describe, for the first time, the structure of the human papovavirus, BKV.

1979.
Israel, M. A.; Chan, H. W.; Rowe, W. P.; and Martin, Malcolm, A. Molecular Cloning of Polyoma Virus DNA in *E. coli.* I. Plasmic Vector System. *Science* 203: 883-87.

1979.
Chan, H. W.; Israel, M. A.; Garon, Claude F.; Rowe, Wallace P.; and Martin, Malcolm, A. Molecular Cloning of Polyoma Virus DNA in *E. coli.* II. Lambda Phage Vector System. *Science* 203: 887-92.

1979.
Israel, M. A.; Chan, H. W.; Martin, Malcolm A.; and Rowe, W. P. Molecular Cloning of Polyoma Virus DNA in *E. coli:* Oncogenicity Testing in Hamsters. *Science* 205: 1140-42.

☐ These three papers describe "risk assessment" experiments to evaluate potential hazards associated with recombinant DNA research. Conclusion: no apparent danger.

1981.
Martin, Malcolm A.; Bryan, T.; Tasheed, S.; and Khan, Arifa S. Identification and Cloning of Endogenous Retroviral Sequences Present in Human DNA. *Proceedings of the National Academy of Sciences, USA* 78: 4892-96.

☐ This paper showed that human DNA contains multiple copies of endogenous retroviral sequences.

1985.
Rabson, A. B.; Daugherty, D. F.; Venkatesan, S.; Boulukos, K. E.; Benn, S. I.; Folks, T. M.; Feorino, P.; and Martin, M. A. Transcription of Novel Open Reading Frames of AIDS Retrovirus During Infection of Lymphocytes. *Science* 229: 1388-90.

☐ This *Science* paper describes, for the first time *all* of the human immunodeficiency virus encoded messenger RNAs.

1986.
Koenig, S.; Gendelman, Howard; Orenstein, J.; Dal Canto, M.; Pezeshkopour, G.; Yungbluth, M.; Janotta, F.; Aksamit, A.; Martin, Malcolm; and Fauci, Anthony. Detection of AIDS Virus in Macrophages in Brain Tissue From AIDS Patients with Encephalopathy. *Science* 233: 1089-93.

☐ This paper was the first to show that HIV resides in macrophages, not in nerve cells, in the brains of AIDS patients with neurological symptoms.

1986.
Gendelman, Howard; Phelps, W.; Feigenbaum, L.; Ostrove, Jeffrey; Adachi, A.; Howley, P.; Khoury, G.; Ginsberg, H.; and Martin, Malcolm. Trans-Activation of the Human Immunodeficiency Virus Long Terminal Repeat Sequence by DNA Viruses. *Proceedings of the National Academy of Sciences, USA* 83: 9759-63.

☐ This paper was the first to show that viruses unrelated to HIV have the capcity to activate a dormant (inactive) copy of the AIDS virus.

Laboratory of Parasitic Diseases (LPD)

Established: 1959. This laboratory existed in many different forms from its founding in 1902.



Chief: Franklin A. Neva, M.D.
1969-

Prior:



Paul P. Weinstein, Sc.D.
1965-68



Leon Jacobs, Ph.D.
1959-65

Previous organization:

Laboratory of Parasite Chemotherapy



Geoffrey M. Jeffery,
Sc.D., M.P.H.
1965-69



J. Robert Coatney,
Ph.D., Sc.D.
1959-65

Laboratory of Tropical Virology



Alexis I. Shelokov, M.D.
1959-64

Laboratory of Tropical Diseases
(1949-59)

Division of Tropical Diseases
(1947-49)

Division of Zoology
(1902-47)



Willard H. Wright,
Ph.D., D.V.M.
1938-58



Maurice C. Hall,
Ph.D., D.V.M.
1936-38



Charles Wardell Stiles, Ph.D.
1902-1931

History:
This laboratory's origins can be traced to the Division of Zoology created in 1902 in the Hygienic Laboratory. Dr. Stiles headed this Division from 1902 until his retirement in 1931, and for the next five years the position remained vacant. In 1936 Dr. Hall was named director; he served until his sudden death in 1938. Under Dr. Wright, who served from 1938 to 1958, the Division of Zoology was renamed the Division of Tropical Diseases (1947) and then the Laboratory of Tropical Diseases (LTD, 1949). When he retired, a reorganization of LTD occurred. Four new laboratories were formed: 1) Laboratory of Parasitic Diseases (LPD); 2) Laboratory of Parasite Chemotherapy (LPC); 3) Laboratory of Germfree Animal Research (LGAR), which had started as a LTD project in 1951 to perform studies on parasites; 4) Laboratory of Tropical Virology (LTV), which was comprised of the Arbo-borne Virus Section of LTD and the Middle American research Unit in the Panama Canal Zone. In a reorganization during the late 1960's, LTV was disbanded, LGAR was transformed into the Laboratory of Microbial Immunity (LMI), and LPC was integrated with LPD into a single Laboratory of Parasitic Diseases under Dr. Neva.

1971.
Gelderman, A. H.; Keister, D. B.; Bartgis, I. L.; and Diamond, L. S. Characterization of the Deoxyribonucleic Acid of Representative Strains of *E. histolytica, E. histolytica*-Like Amebae, and *E. moshkovski. Journal of Parasitology* 57: 906-11.

☐ One of the earliest examples of use of molecular biology to demonstrate important differences in parasites.

1975
Dvorak, J.A.; Miller, L.H.; Whitehouse, W.C.; and Shiroishi, T. Invasionof Erythrocytes by Malarial Merozoites. *Science* 187: 748-50.

☐ Use of low light level video techniques permitted observation that invasion of red cell first involved attachment via specific orientation and later invasion and provided basis for receptor paper.

1975.
Miller, L. H.; Mason, S. J.; Dvorak, J. A.; McGinnis, M. H.; and Rothman, I. K. Erythrocyte Receptors for (*P. knowlesi*) Malaria: Duffy Blood Group Determinants. *Science* 189: 561-563.

☐ First demonstration of receptor mediated invasion of RBCs and explanation for resistance to *P. vivax* in many blacks.

1981.
Udeinya, I. J.; Schmidt, J. A.; Aikawa, M.; Miller, L. H.; and Green, I. Falciparum Malaria-Infected Erythrocytes Specifically Bind to Cultured Human Endothelial Cells. *Science* 213: 555-57.

☐ Provided an *in-vitro* method to study the most important pathophysiologic process in Falciparum malaria.

1984.
Sacks, D. L., and Perkins, P. V. Identification of an Infective Stage of Leishmania Promastigotes. *Science* 223: 1417-19.

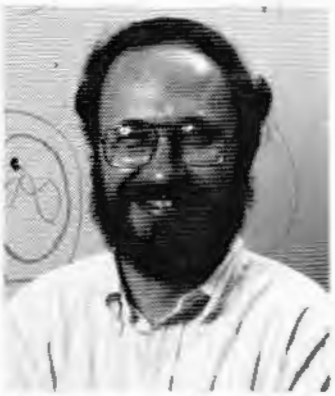
☐ First clear cut demonstration that infective parasites were in stationary instead of log phase of growth, and could be differentiated from noninfective organisms.

1984.
Dame, J. B.; Williams, J. L.; McCutchan, T. F.; Weber, J. L.; Wirtz, R. A.; Hockmeyer, W. T.; Maloy, W. L.; Haynes, J. D.; Schneider, I.; Roberts, D.; Sanders, G. S.; Reddy, E. P.; Diggs, C. L.; and Miller, L. H. Structure of the Gene Encoding the Immunodominant Surface Antigen on the Sporozoite of the Human Malaria Parasite, *P. falciparum. Science* 225: 593-99.

☐ This paper—along with work of the Nussenzweig's—was the first gene cloning of the circumsporozoite protein of a human malaria parasite.

Laboratory of Viral Diseases (LVD)

Established: 1967



Chief: Bernard Moss, M.D., Ph.D.
1984-

Prior:



Wallace P. Rowe, M.D.
1968-83



Robert J. Huebner, M.D.
1967-68

History:
In the 1967 reorganization of intramural research activity, this laboratory was established for Dr. Huebner, who had previously been Chief of the Laboratory of Infectious Diseases. In 1968 Dr. Rowe became Chief of LVD when Dr. Heubner transferred to NCI. In 1984, after Dr. Rowe's death, Dr. Moss left the Laboratory of Biology of Viruses to become Chief of LVD.

1971.
Lowy, Douglas R,; Rowe, Wallace P.; Teich, N.; and Hartley, Janet W. Murine Leukemia Virus: High-Frequency Activation *In Vitro* by 5-Iododeoxyuridine and 5-Bromodeoxyuridine. *Science* 174: 155-56.

☐ This study indicated that the complete MuLV genome is present in unexpressed form iu all cells of the AKR mouse and provided a powerful technique for activation of C-type retrovirus genomes in other systems.

1971.
Pincus, Theodore; Hartley, Janet W.; and Rowe, Wallace P. A Major Genetic Locus Affecting Resistance to Infection with Murine Leukemia Virus. I. Tissue Culture Studies of Naturally Occurring Viruses. *Journal of Experimental Medicine* 133: 1219-33.

☐ These studies established that sensitivity of different laboratory mouse strains to either of the two classes of naturally occurring ecotropic MuLVs is genetically specified and that alleles of the *Fv-1*^{n} gene represent a major determinant of virus spread.

1972.
Rowe, Wallace P. Studies of Genetic Transmission of Murine Leukemia Virus by AKR Mice. I. Crosses with Fv-1^n Strains of Mice. *Journal of Experimental Medicine* 136: 1272-85.

☐ Application of Mendelian genetics to demonstrate that the AKR mouse contains two unliked, autosomal chromosomal loci either of which suffices to induce infectious virus in *Fv-1*^{n} progeny.

1973.
Lewis, Andrew M.; Levine, A. S.; Crumpacker, C. S.; Levin, M. J.; Samaha, R. J.; and Henry, P. H. Studies of Non-Defective Adenovirus 2-Simian Virus 40 Hybrid Viruses. V. Isolation of Additional Hybrids which Differ in Their Simian Virus 40-Specific Biological Properties. *Journal of Virology* 11: 655-64.

☐ By foretelling future developments, this unique study introduced the complexities of the recombinant DNA era by describing the first successful cloning and expression of foreign viral DNAs (in this case overlapping segments of the tumor-inducing region of the genome of SV40) in adenovirus type 2 vectors which were themselves capable of independent replication.

1973.
Kelly, Thomas J., Sr., and Lewis, Andrew M., Jr. Use of Nondefective Adenovirus-Simian Virus 40 Hybrids for Mapping the Simian Virus 40 Genome. *Journal of Virology* 12: 643-52.

☐ This study demonstrated unequivocally, by electron microscopic study of artificially duplexed viral DNAs, that the series of nondefective adenovirus 2- SV40 hybrids described by Lewis *et al.* 1973 did, in fact, contain overlapping segments of the tumor-inducing region of the genome of SV40.

1974.
Chattopadhyay, Sisir K.; Lowy, Douglas R.; Teich, Natalie M.; Levine, Arthur S.; and Rowe, Wallace P. Evidence that the AKR Murine-Leukemia-Virus Genome is Complete in DNA of the High-Virus AKR Mouse and Incomplete in the DNA of the "Virus-Negative" NIH Mouse. *Proceedings of the National Academy of Sciences, USA* 71: 167-71.

☐ This paper represents the first step in establishing the molecular basis of endogenous MuLVs and their expression, establishing that the complete MuLV genome is present in AKR cells as DNA, and that major differences can be detected between high-virus and virus-negative strains of mice.

1976.
Rowe, Wallace P. Leukemia Virus Genomes in the Chromosomal DNA of the Mouse. *Lecture Delivered March 18, 1976 Harvey Lectures Series* 71, 1978, Academic Press.

☐ The concept of endogenous murine leukemia viruses is developed biologically and molecularly, and discussed in the context of the synthesis of theories of genetic and viral etiology of murine leukemia.

1977.
Hartley, Janet, W.; Wolford, Nancy K.; Old, Lloyd J.; and Rowe, Wallace P. A New Class of Murine Leukemia Virus Associated with Development of Spontaneous Lymphomas. *Proceedings of the National Academy of Sciences, USA* 74: 783-92.

— This paper describes the isolation of naturally occurring dual-tropic MuLVs—MCF, or mink cell focus-inducing viruses, characterizing a biologically important new class of MuLV and suggesting its recombinant origin and relationship to murine lymphomagenesis.

1980.
Rowe, Wallace P., and Kozak, C. A. Germ Line Insertions of AKR Murine Leukemia Virus Genomes in AKV-1 Congenic Mice. *Proceedings of the National Academy of Sciences, USA* 77: 4871-74.

☐ This report established that although genetically transferred MuLV loci are stable, during inbreeding of mice congenic for these loci new chromosomal insertions can become established in the germ line.

1985.
Yewdell, J. W.; Bennink, J. R.; Smith, G. L.; and Moss, Bernard. Influenza A Virus Nucleoprotein is a Major Target for Cross-Reactive Anti-Influenza Virus Cytotoxic T Lymphocytes. *Proceedings of the National Academy of Sciences, USA* 82: 1785-89.

— This study illustrated the power of using recombinant vaccinia viruses for determination of cytotoxic T cell targets and was one of the first to demonstrate that internal viral proteins are recognized.

1986.
Rohrman, G.; Yuen, L.; and Moss, B. Transcription of Vaccinia Virus Early Genes by Enzymes Isolated from Vaccinia Virions Terminates Downstream of a Regulatory Sequence. *Cell* 46: 1029-35.

☐ The development of a faithful transcription system opened the way to defining the nucleotide sequences, enzymes and factors involved in initiation and termination of RNA synthesis.

Rocky Mountain Laboratories (RML)

Established: Reorganized in 1979. Attained laboratory status as one of the four components of NMI on November 1, 1948. Prior existence in other forms from 1902, 1921, 1930-31.

Directors, RML before 1979:



Herbert G. Stoenner, D.V.M.
1964-1979



Cornelius B. Philip, Ph.D.
1962-64



Carl Larson, M.D.
1950-62



Ralph R. Parker, Ph.D.
1921-1949

Laboratory Chiefs Since 1979:

Laboratory of Microbial Structure and Function



John L. Swanson, M.D.
1979-

Laboratory of Persistent Viral Diseases



Bruce W. Chesebro, M.D.
1979-

Epidemiology Branch



Willy Burgdorfer, Ph.D.
1982-85 (Acting Chief)



Robert N. Philip, M.D
1979-81 (Acting Chief)

Laboratory of Pathobiology



Claude F. Garon, Ph.D.
1985- (Acting Chief)

History:
The Public Health and Marine Hospital Service in 1902 first became interested in Rocky Mountain spotted fever when it assigned one person from the Hygienic Laboratory (Dr. Julius O. Cobb) to work with the Montana State Board of Health in the Bitterroot Valley of Montana. In the following year, Dr. John F. Anderson was assigned to the study, and in 1904 the group was joined by Dr. Charles Wardell Stiles, who later identified the tick *Dermacentor andersoni*. By 1922 the state had the laboratory in a two-story ex-school building in Hamilton, and it was a recognized field station of the PHS. In 1931 Congress authorized the purchase of the facilities from the state of Montana and the construction of additional buildings. The laboratory continued as a field station under the Division of Scientific Research (as it had from 1922) until 1935 when it was officially named the "Rocky Mountain Laboratory" still under the same administrative jurisdiction. In February 1937, when the Division of Scientific Research merged into the National Institute of Health, the Rocky Mountain Laboratory was made administratively a part of the Division of Infectious Diseases, although retaining its full name. Almost twelve years later, when the National Microbiological Institute was formed, the Rocky Mountain Laboratory attained equal laboratory status with the Institute's other three original components (LID, LTD, DBC). In 1979 the Rocky Mountain Laboratory was reorganized and its name made plural. Dr. Swanson was named Chief of the Laboratory of Microbial Structure and Function and Dr. Chesebro of the Laboratory of Persistent Viral Diseases. No permanent Chief of the Epidemiological Branch was named, but Drs. Philip and Burgdorfer served as Acting Chiefs. Similarly, Dr. Garon has served as Acting Chief of the Laboratory of Pathobiology.

Laboratory of Microbial Structure and Function

1981.
Hackstadt, Ted, and Williams, Jim C. Biochemical Stratagem for Obligate Parasitism of Eukaryotic Cells by *Coxiella burnetii*. *Proceedings of the National Academy of Sciences, USA* 78: 3240-44.

☐ Hackstadt and Williams show that metabolic activity of *Coxiella burnetii* is dependent on acidic conditions, like those in host cell's phagolylosomes; such dependency correlates with this obligate intracellular parasite's survival in a host cell compartment which is usually destructive to bacteria.

1982.
Barbour, A. G.; Tessier, S. L.; and Stoenner, H. G. Variable Major Proteins of *Borrelia hermsii*. *Journal of Experimental Medicine* 156: 1312-24.

☐ Identifies outer membrane proteins that are responsible for the antigenic variation found among relapsing fever causing borreliae.

1985.
Nano, Francis E., and Caldwell, Harlan D. Expression of Chlamydial Genus-Specific Lipopolysaccharide Epitope in *Escherichia coli*. *Science* 228: 742-44.

☐ Nano and Caldwell clone a gene that encodes an enzyme responsible for synthesis of a chlamydia genus-specific lipopolysaccharide antigen.

1986.
Swanson, John; Bergstrom, Sven; Robbins, Kenneth; Barrera, Osmar; Corwin, Dan; and Koomey, Michael J. Gene Conversion Involving the Pilin Structural Gene Correlates with Pilus $^{+} \rightarrow \leftarrow$ Pilus^{-} Changes in *Neisseria gonorrhoeae*. *Cell* 47:267-76.

☐ Shows that similar gene conversion events which utilize different, silent pilin gene sequences account for both on/off switching and antigenic changes of pili by *Neisseria gonorrhoeae*.

1986.
Watkins, Nancy G.; Hadlow, William J.; Moos, Abbie B.; and Caldwell, Harland D. Ocular Delayed Hypersensitivity: A Pathogenic Mechanism of Chlamydial Conjunctivitis in Guinea Pigs. *Proceedings of the National Academy of Sciences, USA* 83: 7480-84.

☐ Explores a guinea pig model of chlamydia-associated conjunctivitis; guinea pigs that spontaneously recover from primary chlamydial infection, though proctected against reinfection by the same agent, exhibit marked ocular hypersensitivity to a common chlamydial antigen.

1987.
MacDonald, Gregory A.; Anacker, Robert, L.; and Garjian, Kareen. Cloned Gene of *Rickettsia rickettsi* Surface Antigen: Candidate Vaccine for Rocky Mountain Spotted Fever. *Science* 235: 83-85.

☐ Reports the cloning of a gene from *Rickettsia rickettsii*; the encoded product constitutes a protective vaccine in guinea pigs against Rocky Mountain spotted fever.

Laboratory of Persistent Viral Diseases

1974.
Chesebro, Bruce; Wehrly, Kathy; Stimpfling, Jack. Host Genetic Control of Recovery from Friend Leukemia Virus-Induced Splenomegaly: Mapping of a Gene Within the Major Histocompatability Complex. *Journal of Experimental Medicine* 140: 1457-67.

☐ This paper was the first to identify the importance of the D region of the H-2 complex in spontaneous recovery from retrovirus-induced leukemia.

1977.
Prusiner, Stanley B.; Hadlow, William J.; Eklund, Carl M.; and Race, Richard E. Sedimentation Properties of the Scrapie Agent. *Proceedings of the National Academy of Sciences, USA* 74: 4656-60.

This paper defined conditions for sedimentation of scrapie agent infectivity and this suggested scrapie was a discrete infectious particle.

1981.
Coe, John E.; Margossian, Sarkis S.; Slayter, Henry S.; and Sogn, John A. Hamster Female Protein: A New Pentraxin Structually and Functionally Similar to C-Reactive Protein and Amyloid P Component. *Journal of Experimental Medicine* 153: 977-91.

This paper showed that hamster female protein, a hamster homologue of the acute phase serum component, C-reactive protein, was a pentraxin under hormonal control.

1983.
Lodmell, Donald L. Genetic Control of Resistance to Street Rabies Virus in Mice. *Journal of Experimental Medicine* 157: 451-60.

This paper demonstrated that recovery from a rabies virus infection was under genetic control in mice and suggested that it might be possible to intervene in this usually fatal infection.

1985.
Bloom, Marshall E., *et al.* Analysis of Aleutian Disease Virus Infection *In Vitro* and *In Vivo:* Demonstration of Aleutian Disease Virus DNA in Tissue of Infected Minks. *Journal of Virology* 53: 696-703.

This paper was the first to demonstrate replicative forms of viral DNA of the Aleutian disease of mink parvovirus in tissues of infected mink.

1985.
Chesebro, Bruce, *et al.* Identification of Scrapie Prion Protein-Specific mRNA in Scrapie-Infected and Uninfected Brain. *Nature* 315: 331-33.

This paper demonstrated that a functional gene for the scrapie associated prion protein occurs in normal as well as scrapie infected animals and that the prion mRNA was not specific for scrapie infection.

1985.
Evans, Leonard, and Lloyd, Miles. Friend and Moloney Murine Leukemia Viruses Specifically Recombine with Different Endogenous Retroviral Sequences to Generate Mink Cell Focus Forming Viruses. *Proceedings of the National Academy of Sciences, USA* 82: 459-63.

This paper indicated that polytropic retroviruses in mice arise by recombination with distinct endogenous viral genes.

1987.
Portis, John., *et al.* Horizontal Transmission of Murine Retroviruses. *Journal of Virology* 61: 1037-44.

This paper demonstrated that an AIDS-like retrovirus could be transmitted venereally between mice of the same or different sexes.

Laboratory of Pathobiology

1972.
Garon, Claude F.; Berry, K. W.; and Rose, James A. A Unique Form of Terminal Redundancy in Adenovirus DNA Molecules. *Proceedings of the National Academy of Sciences, USA* 69: 2391-95.

This paper was the first description of a unique and important sequence arrangement in viruses. Subsequently this type of terminal redundancy was found in a variety of animal virus DNA's and appears to be an important structural feature of many, if not all, linear, duplex genomes.

1981.
Arai, Hideo, and Munoz, John J. Crystallization of Petussigen From *Bordetella Pertussis. Infection and Immunity* 31: 495-99.

This paper reported, for the first time, the crystallization of pertussigen (pertussis toxin) from *Bordetella pertussis.*

1981.
Munoz, John J.; Arai, Hideo; and Cole, Robert L. Mouse-Protecting and Histamine-Sensitizing Activities of Pertussigen and Fimbrial Hemagglutinin from *Bordetella pertussis. Infection and Immunity* 32: 243-50.

This paper demonstrated conclusively that pertussigen (pertussis toxin) is the main mouse protecting antigen in pertussis vaccine.

1981.
Munoz, John J.; Arai, Hideo; Bergman, Robert K.; and Sadowski, Peter L. Biological Activities of Crystalline Pertussigen from *Bordetella pertussis. Infection and Immunity* 33: 820-26.

This paper demonstrated conclusively that a simple crystalline toxin from *Bordetella pertussis* has most of the biological activities found in pertussis vaccine.

1982.

Burgdorfer, Willy; Barbour, Alan G.; Hayes, Stanley F.; Benach, Jorge L.; Grundwaldt, Edgar; and Davis, Jeffery P. Lyme Disease—A Tick-borne Spirochetosis? *Science* 216: 1317-1319.

☐ This article reports the discovery in and isolation from *Ixodes dammini* of the hitherto unknown etiologic agent of Lyme disease in the United States. It also refers to the detection of similar microorganisms (sprochetes) in *I. pacificus* from California and in *I. ricinus* from Switzerland—areas where Lyme disease and related disorders are common. The discovery of Lyme disease spirochete, later named *Borrelia burgdorferi*, has led to intensive epidemiological, clinical, bacteriological, and parasitological investigations of chronic borrelioses throughout the world, but especially in North America and Europe.

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Bloomfield, Arthur L. *A Bibliography of Internal Medicine: Communicable Disease.* Chicago: University of Chicago Press, 1958.

Davis, Dorland J., "Recollections of NIH During World War II," *NIH Alumni Association Newsletter*, October/November 1981, pp. 9-11.

Dyer, R. Eugene, "Medical Research in the United States Public Health Service," *Bulletin of the Society of Medical History of Chicago* 6 (1948): 58-68.

Furman, Bess. *A Profile of the United States Public Health Service, 1798-1948.* Washington, D.C.: U.S. Department of Health, Education, and Welfare, DHEW Publication No. (NIH) 73-369.

Harden, Victoria A. *Inventing the NIH: Federal Biomedical Research Policy, 1887-1937.* Baltimore: Johns Hopkins University Press, 1986.

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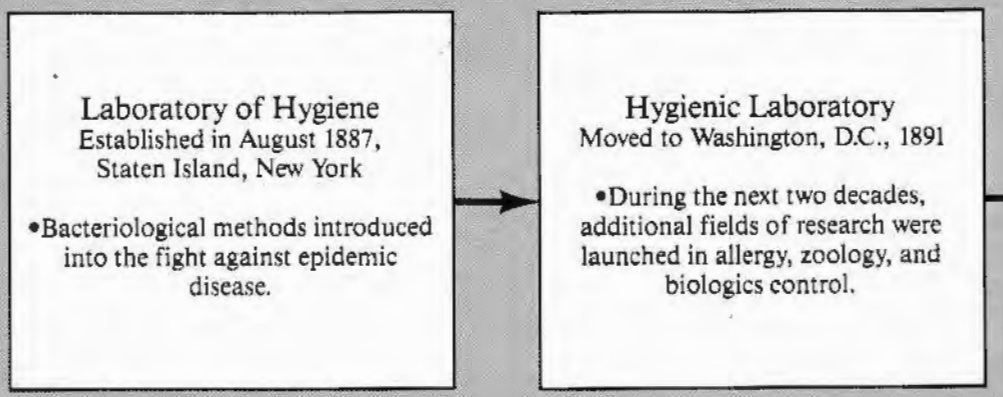
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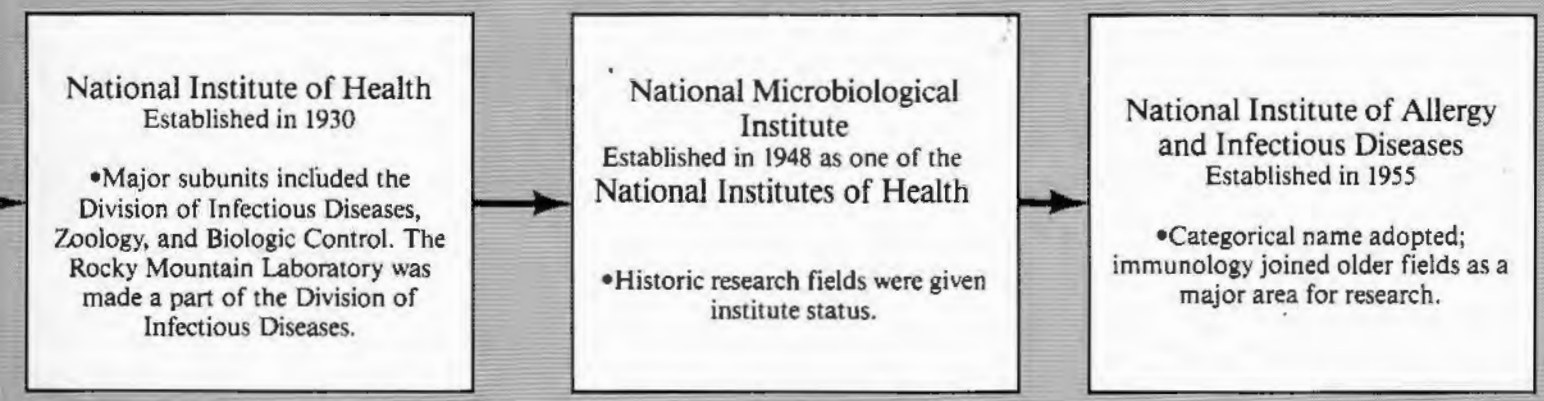
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Stetten, DeWitt and Carrigan, W. T., eds., NIH: *An Account of Research in Its Laboratories and Clinics.* New York: Academic Press, 1984.

Stimson, Arthur H., "A Brief History of Bacteriological Investigations of the U.S. Public Health Service," Supplement No. 141 to *Public Health Reports*, 1938.

Williams, Ralph C. *The United States Public Health Service, 1798-1950.* Washington, D.C.: Commissioned Officers Association of the United States Public Health Service, 1951.


Laboratory of Hygiene
Established in August 1887,
Staten Island, New York
•Bacteriological methods introduced into the fight against epidemic disease.
Hygienic Laboratory
Moved to Washington, D.C., 1891
•During the next two decades, additional fields of research were launched in allergy, zoology, and biologics control.


National Institute of Health
Established in 1930
•Major subunits included the Division of Infectious Diseases, Zoology, and Biologic Control. The Rocky Mountain Laboratory was made a part of the Division of Infectious Diseases.
National Microbiological Institute
Established in 1948 as one of the National Institutes of Health
•Historic research fields were given institute status.
National Institute of Allergy and Infectious Diseases
Established in 1955
•Categorical name adopted; immunology joined older fields as a major area for research.